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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,241,361**, on June 19, 1998, by **McGILL UNIVERSITY**, assignee of M. J. Damha, M. A. Parniak, A. Noronha, C. Wilds, G. Borkow and D. Arion, for "Antisense Oligonucleotide Constructs Based on B-Arabinofuranose and its Analogues".

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BACKGROUND OF THE INVENTION

1. Field of the Invention

It is the primary objective of this invention to provide modified oligonucleotide therapeutic agents to selectively prevent gene transcription and expression in a sequence-specific manner. In particular, this invention is directed to the selective inhibition of protein biosynthesis via antisense strategy using oligonucleotides constructed from arabinonucleotide residues. More particularly this invention relates to the use of antisense oligonucleotides having arabinose sugars to hybridize to complementary cellular messenger RNA and to viral RNA. More particularly this invention relates to the use of arabinonucleic acid strands to hybridize and cleave (via RNaseH activation) complementary RNA. Other applications of this invention relates to the use of antisense oligonucleotides based on arabinonucleotides in combination with RNaseH as laboratory reagents for the sequence specific cleavage and mapping of RNA. This invention further relates to the use of antisense oligonucleotides based on arabinonucleotides in combination with antiviral agents to provide improved inhibition of virus replication. More particularly, this invention relates to the use of antisense oligonucleotides based on arabinonucleotides in combination with dideoxynucleoside antiviral agents to provide improved inhibition of replication of retroviruses such as the human immunodeficiency virus (HIV). This invention also relates to the use of arabinonucleic acid strands to hybridize duplex DNA to form a triple helical complex and thereby block DNA transcription.

2. Description of Prior Art.

(a) The Antisense Strategy. Antisense oligonucleotides have emerged as novel therapeutic agents which can inhibit gene expression in a sequence-specific manner and are currently undergoing clinical-trial evaluations in the treatment of cancer and viral diseases (for reviews see: (i) Uhlman, E.; Peyman, A. *Chem. Rev.* 1990, *90*, 543. (ii) Cook, P.D. *Anti-Cancer Drug Design* 1991, *6*, 585. (iii) Crooke, S.T.

Annu. Rev. Pharmacol. Toxicol. 1992, *32*, 329. (iv) Crooke, S.T.; Lebleu, B. *Antisense Research and Applications*; 1993, pp.579, CRC Press, Boca Raton, FL. (v) Agrawal, S.; Iyer, R.P. *Cur. Op. Biotech.* 1995, *6*, 12.). (vi) DeMesmaeker, A.; Haner, R.; Martin, P., Moser, H. *Acc. Chem. Res.* 1995, *28*, 366. (vii) Crooke, S.T.; Bennett, C.F. *Annu. Rev. Pharmacol. Toxicol.* 1996, *36*, 107). The criteria used in selecting an antisense oligonucleotide as a candidate for potential clinical use require that it exhibit simultaneously a number of desirable characteristics. Three important characteristics for evaluating their potential utility are stability against nucleases, cell-membrane permeability, and the specific binding of the oligomer, by Watson-Crick base pairing, to its complementary sequence (usually mRNA). The formation of a duplex between the antisense oligomer and its target RNA prevents the translation of such RNA by blocking ribosomal reading. This mechanism of action is termed "translation arrest". This, however, is believed to be a minor contributor to the antisense effect. A more predominant mechanism of antisense molecules is the activation of RNaseH, an endogenous enzyme that specifically degrades the RNA message (Walder, R.T.; Walder, J.A. *Proc. Natl. Acad. Sci. USA* 1988, 85, 5011). For example, when an antisense DNA oligonucleotide hybridizes to a mRNA transcript, RNAse H then cuts the mRNA at that site. Antisense oligomers that modulate gene expression by more than one mechanism of action are highly desirable as this increases the potential efficacy of the antisense compound *in vivo*.

(b) Oligonucleotide Analogs. Oligonucleotides containing natural sugars and phosphodiester (PO) linkages are rapidly degraded by serum and intracellular nucleases, thereby rendering them unsuitable as effective therapeutic agents. Chemical strategies to improve nuclease stability include modification of the sugar and base moieties, as well as modification or replacement of the internucleotide phosphodiester linkage. To date, the most widely studied analogues are the phosphorothioate (PS) oligodeoxynucleotides, in which one of the non-bridging oxygen atoms in the phosphodiester backbone is replaced with a sulfur (Eckstein, F. *Ann. Rev. Biochem.* 1985, *54*, 367; see Appendix A-1 for

structures). Several phosphorothioate oligonucleotide analogues are undergoing clinical trial evaluation in the treatment of cancer and viral diseases, and some are moving rapidly towards New Drug Application (NDA) filings (Akhtar, S.; Agrawal, S. *"In vivo studies with antisense oligonucleotides" TiPS* 1997, *18*, 12). Phosphorothioates retain the ability to induce RNaseH and exhibit good nuclease stability properties. However, the therapeutic applications of phosphorothioate molecules is somewhat limited since they form less stable duplexes with the target nucleic acid than do normal phosphodiester oligonucleotides, and exhibit significant nonspecific binding to cellular proteins, thereby reducing the probability of finding and interacting with the target nucleic acid (for a review see: Brach, A.D.; "*A good antisense molecule is hard to find*", *TIBS*, 1998, *23*, 45). Furthermore, phosphorothioate oligodeoxynucleotides are less efficient than the corresponding phosphodiester-linked oligodeoxynucleotides with respect to RNaseH activity (Agrawal, S.; Mayrand, S.H.; Zamenick, P.; Pederson, T. *Proc. Natl. Acad. Sci. USA* 1990, *87*, 1401).

Specificity of action may be improved by developing novel oligonucleotide analogues. Current strategies to generate novel oligonucleotides are to alter the internucleotide phosphate backbone, the heterocyclic base, and the sugar ring, or a combination of these. Alteration or complete replacement of the internucleotide linkage has been the most popular approach, with over 60 types of modified phosphate backbones studied since 1994 (Sanghvi, Y. DNA in "Altered Backbones in Antisense Applications", in *Comprehensive Natural Product Chemistry*, Barton, D.H.R.; Nakanishi, K.; Meth-Coth, O. (eds), 1998, Elsevier Science, Oxford, UK). Apart from the phosphorothioate backbone, only two others have been reported to activate RNaseH activity, i.e., the phosphorodithioate (PS_2) (Seeberger, P.H.; Yen, E.; Caruthers, M.H. *J. Am. Chem. Soc.* 1995, *117*, 1472) and the boranophosphonate backbones (Li, H.; Porter, K.; Huang, F; Shaw, B.R. *Nucleic Acids Res.* 1995, *23*, 4495; Higson, A.P. *et al. Tetrahedron Letters* 1998, *39*, 3899) (Appendix A-1). Because of the higher sulfur content of phosphorodithioate-linked (PS_2) oligodeoxynucleotides, they appear to bind proteins

tighter than the phosphorothioate (PS) oligomers, and to activate RNaseH mediated cleavage with reduced efficiency compared to the PS analogue. Boranophosphonate-linked oligodeoxynucleotides activate RNaseH mediated cleavage of RNA targets, but less well than PO- or PS-linked oligodeoxynucleotides.

Among the reported sugar-modified oligonucleotides most of them contain a five-membered ring, closely resembling the sugar of DNA (D-2-deoxyribose) and RNA (D-ribose). Example of these are α-oligodeoxynucleotide analogs, wherein the configuration of the 1' (or anomeric) carbon has been inverted (Morvan, F.; Rayner, B.; Imbach, J.-L., Chang, D.K.; Lown, J.W. *Nucleic Acids Res.* 1987, *15*, 7027) (Appendix A-2). These analogues are nuclease resistant, form stable duplexes with DNA and RNA sequences, and are capable of inhibiting β-globin mRNA translation via an RNaseH-independent antisense mechanism (Boiziau, C; Kurfurst, R.; Cazanave, C; Roig, V.; Thuong, N.T. *Nucleic Acids Res.* 1991, *19*, 1113). Other examples are xylo-DNA, 2'-O-Me RNA and 2'-F RNA (reviewed in Sanghvi, Y.S.; Cook, P.D. in "Carbohydrate Modifications in Antisense Research", Sanghvi, Y.S.; Cook, P.D. (eds), *ACS Symposium Series*, vol. 580, pp. 1, American Chemical Society, Washington DC, 1994; see Appendix A-2 for structures). These analogues form stable duplexes with RNA targets, however, these duplexes are not substrates for RNaseH. To overcome this limitation, mixed-backbone oligonucleotides ("MBO") composed of either phosphodiester (PO) and phosphorothioate (PS) oligodeoxynucleotide segments flanked on both sides by sugar-modified oligonucleotide segments have been synthesized (Zhao, G. *et al., Biochem. Pharmacol.* 1996, *51*, 173; Crooke, S.T. *et al. J. Pharmcol. Exp. Ther.* 1996, *277*, 923). Among the MBOs most studied to date is the [2'-OMe RNA]-[*PS DNA*]-[2'OMe RNA] chimera . The PS segment in the middle of the chain serves as the RNaseH activation domain, whereas the flanking 2'-OMe RNA regions increases affinity of the MBO strand for the target RNA. MBOs have increased stability *in vivo*, and appear to be more effective than phosphorothioate analogues in their biological activity both *in vitro* and *in vivo*. Examples of this approach incorporating

2'-OMe and other alkoxy substituents in the flanking regions of an oligonucleotide have been demonstrated by Monia *et al.* by enhanced antitumor activity *in vivo* (Monia, P.B.; Johnston, J.F.; Geiger, T.; Muller, M.; Fabbro, D. *Nature Med.* 1996, *2*, 668). Several pre-clinical trials with these analogues are ongoing (Akhtar, S.; Agrawal, S. "In vivo studies with antisense oligonucleotides" *TiPS* 1997, *18*, 12).

The synthesis of oligonucleotides containing hexopyranoses instead of pentofuranose sugars has also been reported (Herdewijn, P. *et al.*, in *"Carbohydrate Modifications in Antisense Research"*, Sanghvi, Y.S.; Cook, P.D. (eds), *ACS Symposium Series,* vol. 580, pp. 80, American Chemical Society, Washington DC, 1994). A few of these analogues have increased enzymatic stability but generally suffer from a reduced duplex forming capability with the target sequence. A notable exception are 6'→4' linked oligomers constructed from 1,5-anhydrohexitol units which, due to their highly pre-organized sugar structure, form very stable complexes with RNA (van Aeroschot, A.C. *et al.*, *Nucleosides & Nucleotides* 1997, *16*, in press). However, none of these hexopyranose oligonucleotide analogues have been shown to elicit RNaseH activity. Recently, oligonucleotides containing completely altered backbones have been synthesized. Notable examples are the peptide nucleic acids ("PNA") with an acyclic backbone (Nielsen, P.E. *in "Perspectives in Drug Discovery and Design"*, vol. 4, pp. 76, Trainor, G.L. (ed.), ESCOM, Leiden, 1996). These compounds have exceptional hybridization properties, and stability towards nucleases and proteases. However, efforts to use PNA oligomers as antisense constructs have been hampered by poor water solubility, self-aggregation properties, poor cellular uptake, and inability to activate RNaseH. Very recently, PNA-[PS-DNA]-PNA chimeras have been designed to maintain RNaseH mediated cleavage via the PS-DNA portion of the chimera (Bergman, F; Bannworth, W.; Tam, S. *Tetrahedron Lett.* 1995, *36*, 6823; Van der Laan, A.C. *et al. Trav. Chim Pays-Bas* 1995, *114*, 295).

(c) Arabinonucleosides and Arabinonucleic Acids (ANA). Arabinonucleosides are stereoisomers of ribonucleosides, differing only in the configuration at the 2'-position of the sugar ring. They have had a substantial impact on chemotherapy and as such they have been extensively used as antiviral and anticancer drugs (for a review, see: Wright, G.E.; Brown, N.C. *Pharmacol. Ther.* 1990, *47*, 447). β-D-Arabinofuranosylcytosine (ara-C) is the most successful nucleoside antileukemic agent and is widely used in combination therapy or at high doses as a single agent to treat patients with acute lymphoblastic and myeloblastic leukemias (Kufe, D.W.; Spriggs, D.R. *Semin. Oncol.* 1985, *12*, 34; Lauer *et al. Cancer* 1987, *60*, 2366). Ara-C has only weak antiviral activity (Stevens, D.A. et al. *N. Engl. J. Med.* 1973, *289*, 873). In contrast, ara-A 5'-monophosphate has substantial activity towards Herpes Simplex Virus (Kaufman, H.E.; Varnell, E.D. *Antimicrob. Agents Chemother.* 1976, *10*, 885). The 5'-monophosphate of 2'-deoxy-2'-fluoro-β-D-arabinofuranosyladenine (2'F-araA-5'MP), is more stable within cells and preliminary investigations indicated that the drug was active against hematologic malignancies (Grever, M.R. *et al. Blood* 1986, *68*, 771). At the cellular level, the major biochemical consequence of arabinonucleosides in treatment is suppression of replicative and repair DNA synthesis. Once ara-C and ara-A are converted intracellularly to their 5'-triphosphate forms (the active metabolites) they are known to work as inhibitors of various DNA polymerases, notably DNA polymerase α, the replicative polymerase in mammalian cells (Lee *et al. Biochemistry* 1980; *19*, 215; Major *et al. Biochem. Pharmacol.* 1982, *31*, 2937; Thompson, H.C.; Kutcha, R.D. *Biochemistry* 1995, *34*, 11198). Another possible mechanism for the inhibition of DNA synthesis by arabinonucleotides involves their misincoporation at the 3'-end of the growing DNA chain, followed by slow rate of addition, or absent rate of addition of the next nucleotide (Momparler R.; *Mol. Pharmacol.* 1972, *8*, 362). For example, Mikita and Beardsley found that araC at the 3' terminus of a DNA chain dramatically reduced the rate of next nucleotide addition for *E. coli* polymerase I, T4 polymerase, HeLa Cell polymerase α_2, and AMV reverse transcriptase (Mikita, T.; Beardsley, G.P. *Biochemistry* 1988, *27*,

4698). Arabinonucleosides can also induce apoptosis in human myeloid leukemia cells lines without being incorporated into DNA (Kuwakado, K. et al. *Biochemical Phar.* 1993, *46*, 1909). Recently, the corresponding 2'-O-allyl arabinonucleosides have been investigated with respect to their biological importance in the inhibition of ribonuclease reductase (Manfredini, S. et al. *Bioorganic and Medicinal Letters* 1997, *1*, 473). While arabinonucleosides have significant antitumor activity, their use is limited by the fact that they are equally toxic to normal rapidly proliferating cell populations. A notable exception is ara-G which is selectively cytotoxic to T-cell lines (Cohen, A.; Lee, J.W.W.; Gelfand, E.W. *Blood* 1983, *61*, 660). The poor water solubility of ara-G and its laborious chemical synthesis have prevented its direct use for treatment of lymphoid cancers and other T-cells disorders, such as autoimmune diseases.

Oligonucleotides constructed from arabinonucleotides ("arabinonucleic acids" or ANA, Appendix A-1) have been under investigation from various different aspects. ANA oligomers have been synthesized as pro-drugs in an attempt to improve the solubility of arabinonucleoside therapeutics. Incorporation of ara-C into DNA strands has also been the focus of research to understand the mechanism of action of this anticancer drug. For example, Beardsley and Mikita reported that the presence of ara-C at the 3'-terminus of a synthetic DNA primer strand results in reduction in the rate of addition of the next nucleotide unit *in vitro* (Mikita, T.; Beardsley, G.P. *Biochemistry* 1988, *27*, 4698). Similarly, a single internucleotide ara-C insertion into an oligomer serving as the template strand hindered the function of T7 RNA polymerase adversely affecting the process of replication *in vitro* (Mikita, T.; Beardsley, G.P. *Biochemistry* 1994, *33*, 9195).

DNA strands containing arabinonucleosides have also been a subject of a number of structural studies. In the crystal, DNA duplexes containing araC adopt a normal B-type double helix with only small conformational perturbations at the araC-dG base pair (Chwang, A.K.; Sundaralingam, M. *Nature* 1973, *243*, 78; Teng, M. *et al. Biochemistry* 1989, *28*, 4923; Gao, Y.-G. *et al.*, *Biochemistry*

1991, *30*, 9922). Mikita and Beardsley prepared DNA/DNA and DNA/RNA duplexes containing a single araC-G base pair to investigate the structural distortions caused by arabinonucleotides. They found that both the DNA duplex and the DNA/RNA hybrid can accommodate araC-dG(rG) base pair with only a moderate and equivalent loss of stability (Mikita, T.; Beardsley, G.P. *Biochemistry* 1994, *33*, 9195). Pfleiderer and co-workers synthesized an all-arabinose oligonucleotide mimicking a transfer RNA molecule (Resmini, M; Pfleiderer, W. *Helv. Chim. Acta* 1993, *76*, 158).

The association properties of uniformly modified oligoarabinonucleotides (ANA) was investigated by Giannaris and Damha and independently by Watanabe and co-workers (Giannaris, P.A.; Damha, M.J. *Can. J. Chem.* 1994, *72*, 909; Kois, P.; Watanabe, K.A. *Nucleic Acids Symposium Series* 1993, *29*, 215; Kois, P. *et al. Nucleosides & Nucleotides* 1993, *12*, 1093). Giannaris and Damha showed that oligomers of either purine or pyrimidine β–arabinonucleosides generally associate with complementary DNA and RNA with thermal stabilities comparable with those of the corresponding DNA strands (Giannaris, P.A.; Damha, M.J. *Can. J. Chem.* 1994, *72*, 909). For example, they showed that (a) an octaarabinoadenylate, ara-A_8 associated with poly ribo-U and poly deoxy-T; the melting temperature of the resulting complex was slightly higher than the corresponding complexes formed by the normal ribo-A_8 and deoxy-A_8 strands; (b) ara-C_8 and ara(UCU UCC CUC UCC C) associated with their complementary RNA strand, *albeit* with lower affinity relative to the corresponding unmodified strands; (c) ara-U_8 did not bind with poly riboA under conditions where ribo-U_8 and deoxy-U_8 formed a complex with poly rA. Giannaris and Damha also reported that replacement of the normal phosphodiester (PO) linkage in ANA oligomers with phosphorothioate (PS) linkages had a severe destabilizing effect; the destabilization was greater than that observed when the PO linkages of a normal DNA strand were replaced with PS internucleotide linkages (Giannaris, P.A.; Damha, M.J. *Can. J. Chem.* 1994, *72*, 909). ANA oligomers displayed some stability against cleavage by snake-venom

phosphodiesterase; however, they were rapidly degraded by nuclease P1, ribonuclease S1 and spleen-phosphodiesterase (Giannaris, P.A.; Damha, M.J. *Can. J. Chem.* 1994, *72*, 909).

Watanabe and co-workers incorporated 2'-deoxy-2'-fluoro-β-D-arabinofuranosylpyrimidine nucleosides (2'-F-ara-N, where N=C, U and T) at multiple positions within a normal DNA chain and evaluated the hybridization properties of such (2'-F)ANA-DNA "chimeras" towards complementary DNA (Kois, P. *et al. Nucleosides & Nucleotides* 1993, *12*, 1093). They found that substitutions with 2'-F-araU and 2'-F-araC had a destabilizing effect on duplex stability, whereas substitution with 2'-F-araT was stabilizing compared to unmodified oligodeoxynucleotide strands. The authors also reported that 2'-F-araT_{11} and 2'-F-araU_{11} oligomers were able to bind to the complementary DNA with equal or slightly better affinity compared to the control dT_{11} (DNA) oligomer. Marquez and co-workers recently evaluated the self-association of a DNA strand in which two internal thymidines were replaced by 2'-F-araT's (Ikeda *et al. Nucleic Acids Res.* 1998, *26*, 2237). They confirmed the findings of Watanabe and co-workers that internal 2'-F-araT residues stabilize significantly the DNA double helix. The association of these (2'-F)ANA-DNA "chimeras" with complementary RNA (the typical antisense target) was not reported.

Recently, Noronha and Damha tested oligonucleotides based on β-D-arabinose for their ability to recognize duplex DNA, duplex RNA and DNA/RNA hybrids (Noronha, A.; Damha, M.J. *Nucleic Acids Res.* 1998, *26*, 2665). A pyrimidine oligoarabinonucleotide was shown to form triple-helical complexes with duplex DNA and hybrid DNA(purine)/RNA(pyrimidine). However, this oligoarabinonucleotide was found to bind with an affinity that was lower relative to the natural pyrimidine oligodeoxynucleotide or oligoribonucleotide controls.

Oligomers constructed from α-arabinofuranosylthymine (α-ara-T) exhibited a large decrease in melting temperature towards complement DNA when compared to the control DNA (β-dT) strand (Adams, A.D.; Petrie, C.R.; Meyer Jr., R.B. *Nucleic Acids Res.* 1991, *19*, 3647). On the other hand, the

duplexes formed between either α-ara-T_{15} or dT_{15} and complementary RNA (poly-rA) were of similar strength. More recently, Wengel and co-workers reported the synthesis and association properties of DNA oligomers containing one and two β-2'-OMe-araT inserts (Gotfredsen, C.H.; Spielmann, P.; Wengel, J.; Jacobsen, J.P. *Bioconjugate Chem.* 1996, *7*, 680). These oligomers showed moderately lowered thermal stabilities towards both single stranded DNA and RNA, compared to unmodified DNA controls. The same authors reported that oligomers constructed from α-2'-OMe-araT units exhibited increased affinity towards the riboadenylate (RNA) target compared to normal DNA controls; however, α-2'-OMe araT strands did not display any advantage relative to the known α-dT oligomers. The susceptibility of the above duplexes to RNase H-mediated cleavage was not investigated.

(d) Activation of RNase H by Antisense Oligonucleotides. As described above, an important mechanism of action of antisense oligonucleotides is the induction of cellular enzymes such as RNaseH to degrade the target RNA (Walder, R.T.; Walder, J.A. *Proc. Natl. Acad. Sci. USA* 1988, *85*, 5011; Chiang *et al. J. Biol Chem.* 1991, *266*, 18162; Monia *et al. J. Biol. Chem.* 1993, *268*, 14514; Giles, R.V., Spiller, D.G.; Tidd, D.M. *Antisense Res. & Devel.* 1994, *5*, 23; Giles *et al. Nucleic Acids Res.* 1995, *23*, 954). RNase H selectively hydrolyzes the RNA strand of a DNA/RNA heteroduplex (Hausen, P.; Stein, H. *Eur. J. Biochem.* 1970, *14*, 279). RNase H1 from the bacterium *Escherichia coli* is the most readily available and the best characterized enzyme. Studies with eukaryotic cell extracts containing RNase H suggest that both prokaryotic and eukaryotic enzymes exhibit similar cleavage properties (Monia *et al. J. Biol. Chem.* 1993, *268*, 14514; Crooke *et al. Biochem J.* 1995, *312*, 599; Lima, W.F.; Crooke, S.T. *Biochemistry* 1997, *36*, 390). *Escherichia coli* RNase H is thought to bind in the minor groove of the DNA/RNA double helix and to cleave the RNA by both endonuclease and processive 3'-to-5' exonuclease activities (Nakamura, H. *et al. Proc. Natl. Acad. Sci. USA* 1991, *88*, 11535; Federoff, O.Y.; Salazar, M.; Reid, B.R. *J. Mol. Biol.* 1993, *233*, 509; Daniher, A.T. *et al. Bioorg. & Med. Chem.* 1997; *5*, 1037). The efficiency of RNase H degradation displays minimal

sequence dependence and is quite sensitive to chemical changes in the antisense oligonucleotide. For example, RNaseH degrades RNA in PS-DNA/RNA hybrids (Gao *et al. Mol. Pharmacol.* 1991, *41*, 223), but not in hybrids containing methylphosphonate-DNA, α-DNA, or 2'-OMe RNA antisense strands. Furthermore, *E. coli* RNaseH binds to RNA/RNA duplexes but cannot cleave them, despite the fact that the global helical conformation of RNA/RNA duplexes is similar to that of DNA/RNA substrate duplexes ("A"-form helices)(Oda *et al. Nucleic Acids Res.* 1993, *21*, 4690). These results suggest that local structural differences between DNA/RNA (substrate) and RNA/RNA duplexes is responsible, at least in part, for substrate discrimination (Oda *et al. Nucleic Acids Res.* 1993, *21*, 4690; Lima, W.F.; Crooke, S.T. *Biochemistry* 1997, *36*, 390). In this regard it is interesting to note that HIV-1 reverse transcriptase (RT)-associated RNaseH cleaves both DNA/RNA and RNA/RNA duplexes; however, cleavage of the latter is at least 30-fold slower and occurs only when RT is artificially arrested (Gotte *et al., EMBO J.* 1995, *14*, 838).

3. Arabinonucleic Acids as Activators of RNaseH Activity- Rationale.

An essential requirement in the antisense approach is that an oligonucleotide or its analogue recognize and bind tightly to its complementary target RNA. The ability of the resulting antisense oligomer/RNA hybrid to serve as a substrate of RNaseH is likely to have therapeutic value by enhancing the antisense effect relative to oligomers that are unable to activate this enzyme. Apart from PS-DNA (phosphorothioates), PS_2-DNA (phosphorodithioates), boranophosphonate-linked DNA, and MBO oligos containing an internal PS-DNA segment, there are no other examples of fully modified oligonucleotides that elicit RNaseH activity. For this reason, and because of the problems encountered with PS-oligonucleotides (e.g., non-antisense effects and potential risk of toxicity), we have designed alternative oligonucleotide analogues that selectively block gene expression through the activation of RNaseH activity. As a starting point, we felt that such analogues should (a) retain the natural β-D-furanose configuration, (b) possess the unmodified phosphate groups for solubility purposes, and (c) be

able to mimic the conformation of DNA strands (e.g., with sugars puckered in the C2'-endo conformation). The latter requirement stems from the fact that the antisense strand of natural substrates is DNA, and as indicated above, its primary structure (and/or conformation) appears to be essential for RNaseH/substrate cleavage. Since the DNA sugars of DNA/RNA hybrids adopt primarily the C2'-endo conformation (Salazar, M.; Champoux, J.J.; Reid, B.R. *Biochemistry* 1993, *32*, 739; Salazar, M.; Federoff, O.Y.; Reid, B.R. *Biochemistry* 1996, *35*, 8126), we were interested in an oligonucleotide analog that favored this conformation. Analogues mimicking the RNA structure (*i.e.*, those that adopt the C3'-endo rather than the C2'-endo conformation) would not be suitable for evoking RNase H activity since it is known that RNA/RNA duplexes are generally not substrate of RNaseH. This prompted us to consider oligomers constructed from arabinonucleotides (i.e., the arabinonucleic acids or ANA). ANA is an stereoisomer of RNA differing only in the stereochemistry at the 2'-position of the sugar ring. Preliminary circular dichroism (CD) experiments conducted in our laboratory strongly suggested that ANA/RNA hybrids adopted a helical structure that was very similar to that of the DNA/RNA substrates (A-form), and as such should be recognized by RNaseH. In addition, X-ray crystallographic studies on ara-C nucleosides and on DNA duplexes containing ara-C indicated that the arabinose sugar adopts the C1'-exo or the C2'-endo conformation typical of DNA strands. Furthermore, examination of molecular models of an A-type ANA/RNA duplex suggested that the β-2'-OH group of the arabinose strand is positioned within the major groove of the hybrid and thus should not interfere with RNase H's binding and catalytic processes. We also considered replacing the β-2'-OH by other electronegative substituents, *e.g.*, fluorine, since strong stereoelectronic effects are expected to stabilize the C2'-endo form (Saenger, W. *Principles of Nucleic Acids Structure*, Cantor, C.R. (ed.), Springer-Verlag, N.Y., 1984; Marquez, V.E.; Lim, B.B.; Barchi, J.J., Jr.; Nicklaus, M.C., "Conformational studies of anti-HIV activity of mono- and difluorodideoxynucleosides", in *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*, Chu, C.K.; Baker, D.C. (eds.), pp.

265-284, Plenum Press, N.Y., 1993). The possibility of an ANA oligomer activating RNaseH has not been reported.

It is the purpose of this invention to provide ANA oligomers and their analogues for sequence specific inhibition of gene expression via association to (and RNaseH mediated cleavage of) complementary messenger RNA. It is also the purpose of this invention to provide ANA oligomers and their analogues that modulate gene expression by binding directly to gene sequences (duplex DNA). Finally, it is the purpose of this invention to provide ANA oligomers and their analogues that direct enhanced incorporation of chain-terminating dideoxynucleoside drugs to specific positions during DNA synthesis by viral and cellular DNA polymerases. This latter strategy will provide therapeutic benefit in treatment of diseases such as HIV infection and rapidly proliferating cancers.

DESCRIPTION OF THE DRAWINGS

FIGURE EX3-1 (A-D): Thermal melting curves of 18-bp heteroduplexes. Oligoarabinonucleotide ANA II and control DNA and RNA oligonucleotides were hybridized to (A) complementary single-stranded RNA, and (B) complementary single-stranded DNA. Oligoarabinonucleotides 2'F-araT_{18} V and 2'F-araA_{18} VI and control DNA and RNA oligonucleotides were hybridized to (C) complementary single-stranded RNA, and (D) complementary single-stranded DNA. The base sequence of the ANA II oligonucleotide is indicated in Table 1. Experimental conditions are provided in Example 3.

FIGURE EX3-2 (A and B). Circular dichroic (CD) spectra of duplexes. (A) This panel clearly shows that the CD spectrum of duplex ANA (II)/RNA matches very well that of the corresponding DNA/RNA duplex (the normal substrate of RNaseH). The CD spectra of the DNA/DNA duplex (of the same base sequence) is very different, and is characteristic of the "B-form" helical conformation. (B) This panel shows the CD spectra of 2'F-araA_{18} (VI)/ rT_{18} and dA_{18}/rU_{18} duplexes, as well as the corresponding DNA/DNA dA_{18}/ dT_{18} duplex. The first two duplexes exhibit a similar CD profiles (i.e., peak pattern and peak position) that is characteristic of the A-helix conformation, whereas the spectrum of the DNA/DNA duplex falls into a pattern that is typical of "B-form" helices. The same conclusions can be reached from the spectra shown in panel (C). The CD spectra of the 2'F-araT_{18} (V)/ rA_{18} duplex displays very similar spectral features of the normal dT_{18} / rA_{18}, also typical of the A-form conformation. The spectra of the duplex dA_{18}/dT_{18} (B-form) is also shown for comparison.

FIGURE EX4-1: Thermal melting curves of triple helical complexes formed by the association of oligoarabinonucleotide VII with DNA/DNA ("DD") and DNA/RNA ("DR") hairpin duplexes. Experimental conditions are provided in Example 4.

FIGURE EX4-2: Gel mobility shift triplex assay under non-denaturing conditions. The figure shows a photograph of a polyacrylamide gel of single stranded oligoarabinopyrimidine VII and target hairpins DNA/DNA VIII ("DD") and DNA/RNA IX ("DR"), and 1:1 ratios of VII:hairpins. The first lane show marker dyes xylene cyanol (XC) and bromophenol blue (BPB). The "VII" lane shows the VII strand, whereas the "DD" (-) shows the DNA/DNA hairpin. The VII:DD triplex is clearly seen in the "DD (+)" lane, which contains a 1:1 molar mixture of VII and "DD". The DNA/RNA hairpin is

visible in the "DR" (-) lane. The triplex VII:DR triplex is clearly visible in the "DR (+)" lane, which consists of a 1:1 mixture of VII and "DR". Gels were visualized by UV-shadowing. Base sequence of single strand VII and hairpins VIII and IX and experimental conditions are given in Example 4.

FIGURE EX5: Oligonucleotides with β-D-arabinose as sugar component elicit RNaseH degradation of complementary target RNA. Defined-sequence 18 nt oligonucleotides were used in these experiments. The antisense oligonucleotide sequence was 5'-AGCTCCCAGGCTCAGATC-3', exactly complementary to a sequence within the U5 region of HIV-1 genomic RNA. The target RNA used was a synthetic ^{32}P-labeled 3',5'-RNA (18 nt), identical to the sequence within the HIV U5 region, and exactly complementary to the sequence of the test oligonucleotides [DNA, DNA-random, ARA (sequence II), 2',5'-RNA and 3',5-RNA]. . The susceptibility of pre-formed oligonucleotide/^{32}P-labeled RNA duplexes to degradation by HIV-1 RT RNase H and by *E. coli* RNase H was assessed. Experimental conditions and interpretations are given in Example 5.

FIGURE EX6: Oligonucleotides with 2'-F-β-D-arabinose as sugar component elicit RNaseH degradation of complementary target RNA. Homopolymeric 18 nt oligonucleotides were used, with complementary 5'-[^{32}P]-labeled rA as target RNA. The susceptibility of pre-formed oligonucleotide/RNA duplexes to degradation by HIV-1 RT RNase H and by *E. coli* RNase H was assessed. For each test compound, the lanes correspond to 0, 5, 10, and 30 minutes incubation with RNase H. "dT" refers to thymidine octadecanucleotide based on 2'-deoxyribose with phosphodiester bonds; "PS-dT" is thymine octadecanucleotide based on β-D-2'-deoxyribose with phosphorothioate bonds; "2'-F-araT" is thymine octadecanucleotide based on β-D-2'-F-arabinose (i.e., sequence V, Table 2); "2'-F-rT" is thymine octadecanucleotide based on β-D-2'-F-ribose (i.e. the 2'-epimer or stereoisomer of V); "rU" is uracil octadecanucleotide based on β-D-ribose; "araU" is uracil octadecanucleotide based on β-D-arabinose (i.e., sequence IV, Table 1). Experimental conditions and interpretations are given in Example 6.

FIGURE EX7: Stability of oligonucleotides with 2'-F-β-D-arabinose as sugar component to degradation by serum nucleases. 18 nt antisense oligonucleotides were labeled with ^{32}P at the 5'-terminus, then incubated with 90% horse serum for varying periods of time, and the resulting nuclease degradation products were electrophoretically resolved and visualized by autoradiography. "dT",

thymine octadecanucleotide based on β-D-2'-deoxyribose with normal (PO) phosphodiester bonds; "2'-F-araT", thymine octadecanucleotide based on 2'-F-β-D-arabinose (i.e., sequence V, Table 2). For each oligonucleotide, the samples illustrated are those at 0, 5, 10, 15, 20 and 30 minutes of incubation.). Experimental conditions and interpretations are given in Example 7.

FIGURE EX8: Nonspecific interaction of oligonucleotides with cellular proteins. 18 nt thymine oligonucleotides based on β-D-2'-deoxyribose with normal (PO) phosphodiester bonds ("dT"), β-D-2'-deoxyribose with phosphorothioate bonds ("PS-dT"), or β-D-2'-F-arabinose ("aFT", i.e., sequence V, Table 2) were labeled with ^{32}P at the 5'-terminus, then incubated with mouse liver crude extract proteins. The resulting free and protein-bound oligonucleotides were separated by non-denaturing gel electrophoresis and visualized by autoradiography. B: position of protein-bound oligonucleotide. F: position of unbound free oligonucleotide. Intermediate bands correspond to oligonucleotide that dissociated from protein during the electrophoresis. Lanes 1, 5, 9: no cell protein. Lanes 2, 6, 10: undiluted cell protein. Lanes 3, 7, 11: cell protein diluted 1:10. Lanes 4, 8, 12: cell protein diluted 1:100.

FIGURE EX9-1: Effect of oligonucleotides with 3'-terminal β-D-arabinose on chain termination by 2',3'-dideoxynucleotides during DNA synthesis catalyzed by HIV-1 reverse transcriptase. HIV-1 RT-catalyzed DNA synthesis was carried out using a synthetic 30 nt RNA template and synthetic 18 nt DNA primers, exactly complementary to the 18 nt at the 3'-end of the template RNA. Two different primers were used, differing only in the sugar component of the 3'-terminal nucleotide, one with a 2'-deoxyribose at the 3'-terminus (i.e., 5'-dAGCTCCCAGGCTCAGATC-3', or "D18") and the other with β-D-arabinose at the 3'-terminus (*i.e.*, 5'-dAGCTCCCAGGCTCAGATaraC-3', abbreviated as "D17aC"). The 18 nt oligonucleotides were labeled with ^{32}P at the 5'-end and mixed with an equivalent concentration of RNA transcript to allow formation of duplex template/primers (T/P). The Figure illustrates DNA polymerization products formed by HIV-1 RT after addition of dATP+dCTP+dGTP+dTTP to a final concentration of 25 μM each, in the absence or the presence of ddCTP (10 μM). See Example 9 for details.

FIGURE EX9-2: Ratio of chain-termination products at n+4 compared to n+3. The data are plotted from densitometric traces of the appropriate bands in FIGURE EX9-1.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides the first examples of uniformly sugar-modified oligonucleotides that elicit RNaseH activity. RNaseH has been implicated in the major mechanism of action of antisense therapeutics. These oligomers are composed of arabinonucleosides (ANA oligomers), which are stereoisomers of natural ribonucleosides differing only in the stereochemistry at the 2'-position of the sugar ring, To date, only the natural DNA structure and phosphorothioate and dithioate backbones have been reported to evoke RNase H activity. Also provided are oligonucleotides based on arabinonucleotides that bind to duplex DNA.

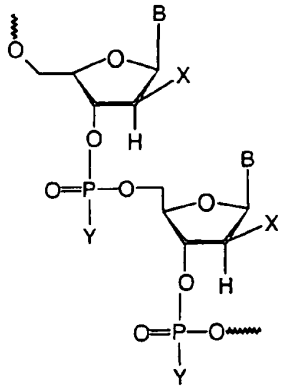
In another aspect of the invention, oligodeoxynucleotides containing a single arabinonucleoside (e.g., araN or 2'-F araN) at or near the 3'-end were prepared and targeted to genomic HIV-1 RNA. These novel DNA-ANA "chimeras" were able to serve as polymerization primers for DNA synthesis catalyzed by the virally encoded reverse transcriptase (RT). However, this DNA synthesis was consistently "paused" at a position +4 nucleotides downstream from the position of the arabinonucleoside in the synthetic DNA-ANA "chimera". This pausing was not seen when a simple DNA strand of identical sequence was employed. The artificial pausing induced by DNA-ANA chimeras resulted in enhanced incorporation of the 2',3'-dideoxynucleoside complementary to the template base at the position +4 nucleotides downstream from the position of the arabinonucleoside in the synthetic DNA-ANA "chimera", when compared to the incorporation of the same 2',3'-dideoxynucleoside in reactions primed by an identical sequence simple DNA strand.

In summary, our experiments establish that ANA oligomers serve as excellent models of antisense agents that have enhanced resistance to the action of degradative nucleases, bind to RNA through duplex formation, elicit RNase H activity, and direct specific incorporation of chain-terminating nucleotides. As such, they should serve as therapeutics and/or valuable tools for studying and controlling gene expression in cells and organisms.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to oligonucleotides based on β-D-arabinose and its derivatives and the therapeutic use of such compounds. It is the object of the present invention to provide a new oligonucleotide analogue that hybridizes to complementary nucleic acids which may be mRNA, viral RNA (including retroviral RNA), or duplex DNA for the purpose of inhibiting gene transcription and expression. More particularly this invention relates to the use of arabinonucleic acid strands to cleave complementary RNA via RNaseH activation. Other applications of this invention relates to the use of antisense oligonucleotides based on arabinonucleotides in combination with RNaseH as laboratory reagents for the sequence specific cleavage and mapping of RNA. This invention further relates to the use of antisense oligonucleotides based on arabinonucleotides in combination with antiviral agents to provide improved inhibition of virus replication. More particularly, this invention relates to the use of antisense oligonucleotides based on arabinonucleotides in combination with dideoxynucleoside antiviral agents to provide improved inhibition of replication of retroviruses such as the human immunodeficiency virus (HIV).

The oligonucleotides of this invention may be represented by the following formula (I):



Where B includes but it is not necessarily limited to a common purine or pyrimidine base such as adenine, guanine, cytosine, thymine, and uracil. The sugar is β-D-arabinofuranose, its mirror image enantiomer β-L-arabinofuranose, and the corresponding carbocyclic sugars (i.e., in which the ring oxygen at position 4' is replaced by a methylene or CH_2 group). X at the 2' position of the sugar ring includes but it is not necessarily limited to a halogen (fluorine, chlorine, bromine, iodine), hydroxy, alkyl, allyl, amino, aryl, alkoxy, and azido. Y at the internucleotide phosphate linkage includes but it is not necessarily limited to oxygen, sulfur, methyl, amino, alkylamino, dialkylamino, methoxy, and ethoxy. The ANA oligomers may also include modified sugars in part of the oligomer. The oligonucleotides may also include the 2'-deoxy-2',2'-difluoro-β-ribofuranose sugar (D or L configuration) in part or all of the oligomer (this structure is obtained by replacing the 2'-H atom in formula I with a fluorine atom, thus providing an oligonucleotide containing *two* fluorine atoms at carbon 2'). The ANA oligomers of this invention contains a sequence that is complementary to a specific sequence of a mRNA, or genomic viral RNA, such that the oligonucleotide can specifically inhibit protein biosynthesis, or virus replication (reverse transcription), respectively. A complementary target may also be duplex or single stranded DNA, such that the arabinonucleotide strand can specifically inhibit DNA replication and/or transcription. Partial modifications to the oligonucleotide directed to the 5' and/or 3'-terminus, or the phosphate backbone or sugar residues to enhance their antisense properties are within the scope of the invention.

A preferred group of oligonucleotides useful in this invention, are those wherein B is a natural base (adenine, guanine, cytosine, thymine, uracil); the sugar moiety is β-D-arabinofuranose; X is fluorine; Y is oxygen since these modifications give rise to oligomers that exhibit high affinity for single stranded RNA, single stranded DNA, and duplex DNA. In addition, these oligomers have been shown to meet the requirements necessary for antisense therapeutics. For example, they activate RNaseH activity

better than phosphorothioate-linked oligodeoxynucleotide, and as such may be potentially more effective *in vivo*.

The free β-D-arabinose pyrimidine (araU, araC) nucleoside monomers may be prepared from the corresponding ribonucleosides in good yields, and can be further elaborated to the corresponding 5'-O-monomethoxytrityl-2'-O-acetyl-3'-O-(β-cyanoethylphosphoramidite) derivatives suitable for solid-phase oligonucleotide synthesis (Giannaris, P.A.; Damha, M.J. *Can. J. Chem.* 1994, *72*, 909). The corresponding araA nucleoside is commercially available, and can be prepared readily from riboadenosine (via oxidation of the 2'-OH group and reduction of the 2'-keto group with a hydride source, e.g., Robins, M.J. *et al.* in *"Nucleosides, Nucleotides and their Biological Applications"*, Rideaut, J.L.; Henry, D.W.; Beacham III, L.M. (eds.), pp. 279, Academic Press, Inc., 1993). The corresponding ara-G monomer can be prepared by the method of Pfleiderer and co-workers (Resmini, M..; Pfleiderer, W. *Helv. Chim. Acta* 1994, *77*, 429). The 3'-O-(β-cyanoethyl-N,N-diisopropylphosphoramidite) derivatives of 5'-MMT-2'-deoxy-2'-fluoro-β-D-arabinonucleosides (2'F-ara-C, 2'F-ara-A, and 2'F-ara-T) may be synthesized following published procedures (Tann, C.H.; Brodfuehrer, P.R.; Brundidge, S.P.; Sapino, C. Jr.; Howell, H.G. *J. Org. Chem.* 1985, *50*, 3644; Howell; H.G.; Brodfuehrer, P.R.; Brundidge, S.P.; Benigni, D.A.; Sapino, C., Jr. *J. Org. Chem.* 1988, *53*, 85; Kois, P.; Tocik, Z.; Spassova, M.; Ren, W.-Y.; Rosenberg, I.; Farras Soler, J.; Watanabe, K.A. *Nucleosides & Nucleotides* 1993, *12*, 1093).

The protected arabinonucleoside monomers can be attached to the solid support by known methods. In a preferred embodiment, the solid support is long-chain alkylamine controlled pore glass, and the procedure of Damha *et al.* is used for its derivatization (Damha *et al. Nucleic Acids Res.* 1990, *18*, 3813).

The ANA oligomers of this invention (constructed from either β-D-arabinose or 2'-deoxy-2'-fluoro-β-D-arabinose) exhibit a number of desirable antisense properties:

(1) They were found to bind to and cleave single stranded RNA by activating RNaseH. Circular dichroism studies in solution showed that DNA/RNA hybrids (the natural substrate of RNase H) and ANA/RNA duplexes adopt a very similar helical structure that falls within the "A"-conformational family. The ability of RNaseH to degrade RNA in the ANA:RNA duplexes may be due, at least in part, to (a) the similarity of the structure of ANA/RNA to that of DNA/RNA duplexes, and (b) the fact that the 2'-substituent of the sugar ring is located in the major groove, where it does not interfere in RNase H's binding and catalytic processes. The 2'-fluorinated ANA derivatives in particular were found to have excellent affinity towards RNA targets, compared to normal DNA and phosphorothioate oligodeoxynucleotide strands.

An oligonucleotides based on β-D-arabinose and containing four nucleobases (U, C, A and G) was found to hybridize to complementary RNA but not complementary single stranded RNA. This property suggests that these oligomers may be useful for targeting retroviral genomic RNA to inhibit early stages of virus replication, including reverse transcription. This high level of RNA specificity has previously been reported for other types of oligonucleotide analogs (e.g., 2',5'-linked RNA and 2',5'-linked DNA; Giannaris, P.A.; Damha, M.J., *Nucleic Acids Res.* 1993, *20*, 4742; Alul, R.; Hoke, G.D. *Antisense Res. Dev.* 1995, *5*, 3), however, none of these oligonucleotides elicit RNaseH activity.

Pyrimidine oligonucleotides constructed from 2'-deoxy-2'-fluoro-β-D-arabinonucleoside units were also found to hybridize to duplex DNA and DNA/RNA hybrids via triplex helix formation. The thermal stability of these triplexes are significantly higher than those formed by normal oligodeoxynucleotides. These results were unexpected given that the β-D-arabinose series produces triplexes with only modest stability (Noronha, A.; Damha, M.J. *Nucleic Acids Res.* 1998, *26*, 2665).

(2) Results from metabolic stability studies indicate that the arabinose modification, particularly the 2'-F derivatives, confers greater resistance to degradation by both serum and cellular nucleases compared with natural strands (PO-DNA), although less than to phosphorothioate (PS-DNA)

derivatives. However, the latter molecules show very high nonspecific binding to cellular proteins (and likely to serum proteins), whereas the arabinose modified oligomers show little nonspecific protein binding. This decreased nonspecific protein binding results in a significantly improved interaction of arabinooligonucleotides (ANA) with target RNA in the presence of cell proteins compared to the phosphorothioate analogs.

(3) Oligodeoxynucleotides possessing an arabinose sugar at the 3'-end of the chain (e.g., 5'-DNA-araC-3') are able to serve as primers for HIV RT-catalyzed DNA synthesis. Unexpectedly, it was noted that this priming results in an unusual pattern of polymerization, that is, significant pausing was noted after the addition of 4 nucleotides (n+4 product); this product was noted only with the 5'-DNA-araC-3' oligonucleotide. No significant amounts of shorter (n+1...3) products were observed. The appearance of the n+4 product is sequence independent, since it is noted with several different oligomers possessing a 3'-terminal arabinonucleotide (e.g., (a) 5'-DNA-araC-3', (b) 5'-DNA'-2'F-araC-3', etc, where DNA and DNA' represent different sequences). Previous studies had postulated that incorporation of ddN chain terminators might occur more frequently at RT polymerization pause sites (Arts, E.J.; Wainberg, M.A. *Antimicrobial Agents and Chemotherapy*, 1994, *38*, 1008). We found that the incorporation of ddN complementary to the template base at the n+4 position was enhanced relative to incorporation of that same ddN at complementary sites other than the n+4 site. This ddN incorporation in reactions primed by the 5'-DNA-araN-3' oligonucleotide is substantially increased compared to that in reactions primed by normal DNA strand, suggesting that appropriate combinations of 5'-DNA-araN-3' with ddN may be of therapeutic benefit in treatment of HIV infection.

These properties combined establish that ANA oligomers serve as excellent models of antisense agents that have enhanced resistance to the action of degradative nucleases, bind to RNA and single stranded DNA through duplex formation, bind to duplex DNA through triplex formation, elicit RNase H activity, and direct specific incorporation of chain-terminating nucleotides. Consequently, antisense

oligonucleotide constructs containing arabinose and their analogues should serve as therapeutics and/or valuable tools for studying and controlling gene expression in cells and organisms.

The following examples are given by way of illustration of the present invention. The examples are not intended in any way to limit the scope of the invention.

EXAMPLE 1

Preparation of Oligonucleotides containing β-D-Arabinofuranoses

Oligoarabinonucleotides (Formula I; X= OH, Y= O^-) were synthesized using standard phosphoramidite chemistry and 3'-ara-C(Bz)-long-chain alkylamine controlled pore glass solid support (lcaa-CPG; 500 Å; 1 μmol scale). The required monomers, namely 5'-MMT-2'-OAc-3'-O-(β-cyanoethyl-N,N-diisopropylphosphoramidite) derivatives of ara-A(Bz), ara-C(Bz) and ara-U were synthesized by the method of Damha *et al.* (Damha, M.J.; Usman, N.; Ogilvie, K.K., *Can. J. Chem.* 1988, *67*, 831; Giannaris, P.A.; Damha, M.J.; *Can. J. Chem.* 1994, *72*, 909). The corresponding ara-G (N^2-*i*-Bu, O^6-NPE) monomer was prepared by a modification of the procedure of Resmini *et al.* (Resmini, M.; Pfleiderer, W. *Helv. Chim. Acta* 1994, *77*, 429). Thus, the monomers were dissolved to 0.12 M in anhydrous acetonitrile. Prior to chain assembly, the support (1 μmol) was treated with the capping reagents, acetic anhydride/N-methylimidazole/4-dimethylaminopyridine (Damha, M.J.; Ogilvie, K.K. in *Methods in Molecular Biology, 20, Protocols for Oligonucleotides and Analogs: Synthesis and Properties*, Agrawal, S. (ed.), pp. 81, The Humana Press, Inc. Totawa, N.J., 1993). Chain assembly of sequences was carried out using an Applied Biosystem DNA synthesizer (Model 381A) as follows: (i) detritylation: 3% trichloroacetic acid in dichloroethane delivered in 100 s (+ 40 s 'burst') steps. The eluate from this step was collected and the absorbance at 478 nm (MMT+, arabino sequences) measured to determine the average coupling reaction yield (ca. 90%); (b) nucleoside phosphoramidite coupling time of 7.5 min; (c) capping: 1:1 (v/v) of acetic anhydride/collidine/THF 1:1:8 (solution A) and 1-methyl-1*H*-imidazole/THF 16:84 (solution B) delivered in 62 s + 35 s "wait" steps; (d) oxidation: 0.05M iodine in THF/water/pyridine 7:2:1, delivered in 20 s + 35 s "wait" step. The 5'-terminal trityl group was removed by the synthesizer and the oligomers were then removed from the support and deprotected by treatment of the CPG with a solution containing concentrated ammonium hydroxide/ethanol (3:1 v/v, 1 mL) for two days at room temperature. The ammonium

hydroxide/ethanol solution was evaporated and the crude product purified by preparative polyacrylamide gel electrophoresis (PAGE) followed by gel filtration (desalting) on a Sephadex G-25 column. For sequences containing ara-G units, it was necessary to subject the partially protected oligomer to an additional step; that is, following the ammonia treatment and evaporation step, the oligomer was treated with a solution of 1M tetra-*n*-butylammonium fluoride (50 mL, r.t., 16 h) in THF. This step cleaves the *p*-nitrophenylethyl protecting group at the O6-position of guanine residues. This solution is then quenched with water (1 mL) and desalted via size exclusion chromatography (Sephadex G-25 column). Purification is then carried out by gel electrophoresis as described above. The yield, base sequence , and molecular weight (as determined by MALDI-TOF mass spectrometry) of the oligomers synthesized are given in TABLE 1.

Presently only the 5'-DMT, 2'-OAc, ara-C (Bz) 3'-phosphoramidite derivative and 3'-ara-C (Bz) long-chain alkylamino controlled pore glass (lcaa-CPG) are commercially available. Of the free (unprotected) nucleosides, only ara-C, ara-U and ara-A are commercially available.

EXAMPLE 2

Preparation of Oligonucleotides containing 2-Deoxy-2-Fluoro-β-D-Arabinose sugars.

Oligoarabinonucleotide synthesis (Formula I; X= F, Y = O^-) was performed on an Applied Biosystem DNA synthesizer (model 381A) using the phosphoramidite approach. Oligomers were prepared on a 1.0 μmol scale using lcaa-CPG solid support bearing 3'-terminal 2'-deoxy-2'-fluoro-β-D-arabinonucleosides. Coupling yields ranged from 60 to 100% (average ca. 80%) as monitored by the release of the MMT cation. The required 3'-O-(β-cyanoethyl-N,N-diisopropylphosphoramidite) derivatives of 5'-MMT-2'-deoxy-2'-fluoro-β-D-arabinonucleosides (ara-C, ara-A, and ara-T) were synthesized by published procedures (Tann, C.H.; Brodfuehrer, P.R.; Brundidge, S.P.; Sapino, C. Jr.; Howell, H.G. *J. Org. Chem.* 1985, *50*, 3644; Howell; H.G.; Brodfuehrer, P.R.; Brundidge, S.P.;

Benigni, D.A.; Sapino, C., Jr. *J. Org. Chem.* 1988, *53*, 85; Kois, P.; Tocik, Z.; Spassova, M.; Ren, W.-Y.; Rosenberg, I.; Farras Soler, J.; Watanabe, K.A. *Nucleosides & Nucleotides* 1993, *12*, 1093). Thus, the monomers were dissolved to 0.10 M in anhydrous acetonitrile. Prior to chain assembly, the support (1 μmol) was treated with the capping reagents, acetic anhydride/N-methylimidazole/4-dimethylamino pyridine (Damha and Ogilvie). Chain assembly of sequences was carried out as follows: (i) detritylation: 3% trichloroacetic acid in dichloroethane delivered in 100 s (+ 40 s 'burst') steps. (b) nucleoside phosphoramidite coupling time of 10 min; (c) capping of 5'-hydroxyl groups: 1:1 (v/v) of acetic anhydride/collidine/THF 1:1:8 (solution A) and 1-methyl-1*H*-imidazole/THF 16:84 (solution B) delivered in 62 s + 35 s "wait" steps; (d) oxidation of phosphite triester linkage: 0.05M iodine in THF/water/pyridine 7:2:1, delivered in 20 s + 35 s "wait" step. The 5'-terminal trityl group was removed by the synthesizer and the oligomers were then removed from the support and deprotected by treatment of the CPG with a solution containing concentrated ammonium hydroxide/ethanol (3:1 v/v, 1 mL) for one day at room temperature. The ammonium hydroxide/ethanol solution was evaporated and the crude product purified by preparative polyacrylamide gel electrophoresis (PAGE) followed by gel filtration (desalting) on a Sephadex G-25 column. The yield, base sequence , and molecular weight (as determined by MALDI-TOF mass spectrometry) of the oligomers synthesized are given in TABLE 2.

EXAMPLE 3

Association Properties of Uniformly Modified Oligonucleotides possessing β-D-Arabinose and β-D-2'-Fluoro-2'-Deoxyarabinose Sugars. Binding to single stranded DNA and RNA Targets

The ability of oligonucleotides to hybridize to single-stranded nucleic acids to give a double-helical complex is crucial for their use as antisense therapeutic agents. The formation of such a complex involves stacking and hydrogen bonding interactions between the base chromophores, a process which is accompanied by a reduction in UV absorption ("hypochromicity"). When the

temperature of the solution containing the double-helical complex is gradually raised, the hydrogen bonds break and the duplex dissociates into single strands. This reduces the amount of base-base interactions and hence leads to a sudden increase of the UV absorbance. The temperature at which the double-helical complex dissociates, or more precisely, the point at which half the population exists as complex and the remaining half as single strands, is termed the "melting temperature" (T_m). Thus a common technique used in nucleic acid chemistry to investigate duplex formation (and its strength) involves mixing equimolar amounts of the strand of interest together, incubating at low temperature to allow strands to anneal, and then observing the UV-absorption at 260 nm (absorption maxima) as a function of temperature. The result is an absorbance versus temperature plot, or sigmoidal "melt profile" or "melting curve" from which the T_m (the midpoint of the raise of the melt curve) is calculated (Wickstrom, E.; Tinoco, I. Jr. *Biopolymers* 1974, *13*, 2367). Circular dichroism (CD) is another poweful optical technique for the study of nucleic acid structure and conformation. The CD spectrum usually includes a region of rapid change (Cotton effects) with respect to wavelength (200-350 nm region). The signs, absolute intensity and position of the Cotton effects are particularly sensitive to chemical composition and three-dimensional structure of the nucleic acid complexes. CD measurements can therefore be applied to determine global helical conformation (or helix type) as well as to investigate structural changes (e.g., helix-to-coil transitions) as a function of temperature (Bloomfield, V.A.; Crothers, D.; Tinoco, Jr., I. *"Physical Chemistry of Nucleic Acids"*, Harper and Row, N.Y., 1974; Ts'o, P.O.P. (ed.), "Basic Principles in Nucleic Acid Chemistry", vol. 1 and 2, Academic Press, N.Y., 1974).

The binding properties of oligoarabinonucleotides **II**, **V**, and **VI** (for sequences see Tables 1 and 2) with complementary DNA and RNA single strands were evaluated in a buffer containing 140 mM KCl, 1 mM $MgCl_2$, 5 mM Na_2HPO_4 (pH 7.2), which is representative of intracellular conditions (Alberts, B. *Molecular Biology of the Cell*, pp. 304, Garland, N.Y., 1989). Molar extinction

coefficients for oligoarabinonucleotide strands were calculated using the nearest-neighbor approximation, and were assumed to be the same as those of normal strands (Puglisi, J.D.; Tinoco, I. Jr. Methods in Enzymology, Dahlberg, J.E.; Abelson, J.N. (eds.), *180*, pp. 304, Academic Press, S.D., 1989). Thermal denaturation curves were acquired at 260 nm from 5 °C to 90 °C (rate of heating: 0.5 °C/min), at a concentration of approximately 2.8 μM of each strand. Melting temperatures (T_m) were calculated from first-derivative plots of absorbance versus temperature. Thermal denaturation curves of the heteroduplexes are shown in FIGURE EX3-1 (A and B).

The results (Figure EX3-1A) show that the arabinonucleotide of mixed base sequence **II** has the ability to form a stable heteroduplex with its RNA complements, exhibiting a T_m of 44 °C, compared to 66 °C for the corresponding natural DNA/RNA heteroduplex. Interestingly, a 1:1 mixture of **II** and its DNA complement showed a much weaker and broader transition suggesting that, under the conditions used, **II** does not bind to single stranded DNA (Figure EX3-1B). The data also show that interaction of 2'-F-oligoarabinonucleotides **V**, and **VI** with complement RNA results in the formation of heteroduplexes that are of superior thermal stability relative to the complexes formed by the natural oligodeoxynucleotide strands. For example, the T_m of 2'-F-araA_{18} (**VI**)/ rU_{18} heteroduplex was 30.2 °C, compared to 25.4 °C for the natural dA_{18}/ rU_{18} heteroduplex. Similarly, the T_m of the 2'-F-araT_{18}/ rA_{18} heteroduplex was 43.9 °C, which represents an increase in T_m of ca. 5 °C relative to the natural dT_{18} / rA_{18} heteroduplex (T_m 39 °C) (Figure EX3-1C). In contrast to the behaviour observed for sequence **II** (which exhibited selective binding to RNA), oligonucleotides **V** and **VI** bind strongly to both single stranded complementary DNA and RNA. In fact, 2'-F-araA_{18} (**VI**) formed a more stable heteroduplex with single stranded complementary DNA (dT_{18}) than with RNA (rU_{18}), i.e., T_m 63.3 °C and 30.2 °C, respectively (Figures EX3-1C and D). This amounts to a binding selectivity of ΔT_m = +33 °C . The selective binding of 2'-F-araA_{18} to single stranded DNA (over RNA) was also observed for the natural dA_{18} strand, although in this case the selectivity observed was less (ΔT_m = +20 °C).

As shown in FIGURE EX3-2(A-C), the CD spectra of the heteroduplexes II/RNA, V/RNA and VI/RNA closely resembled those of the corresponding DNA/RNA duplexes, suggesting that all of these complexes share the same helical conformation. The spectral features observed are characteristic of a "A"-type helix, a structure that appears to be important in the recognition of DNA/RNA substrates by RNase H (Lima, W.F., Crooke, S.T. *Biochemistry* 1997, *36*, 390). The fact that the oligoarabinonucleotid/RNA heteroduplexes are substrates of RNaseH (see Examples 5 and 6) is fully consistent with this notion.

EXAMPLE 4

Association Properties of Oligonucleotides possessing 2'-Deoxy-2'-Fluoro-β-D-Arabinose Sugars. Binding to DNA Duplexes and DNA/RNA Hybrids

To study the interaction between oligomers of 2'-deoxy-2'-fluoroarabinonucleotides and DNA/DNA and DNA/RNA duplexes, the experimental design of Roberts and Crothers was adopted (Roberts, R.W.; Crothers, D.M. *Science* 1992, *258*, 1463). The target duplexes are the following purine-pyrimidine hairpins (VIII and IX):

DNA/DNA (VIII)

```
5'-GGAGAGGAGGGA T
                 T
                 T
3'-CCTCTCCTCCCC T
```

DNA/*RNA* (IX)

```
5'-GGAGAGGAGGGA T
                 T
                 T
3'-CCUCUCCUCCCC T
```

Triplex-helix formation can occur when an oligonucleotide binds in the major groove of the targeted duplexes (Le Doan, T. *et al. Nucleic Acids Res.* 1987, *238*, 645; Moser; H.E.; Dervan, P.B. *Science* 1987, *238*, 645). The oligoarabinopyrimidine strand (VII) , i.e., 2'-F-ara(CCTCTCCTCCCT,

containing 2'-deoxy-2'-fluoro-arabinose), was used to assess triple helix formation with the above hairpin duplexes. For the purpose of comparisons, the association of the corresponding oligodeoxyribopyrimidine ("DNA", 2'-deoxy-β-D-ribose) and oligoarabinopyrimidine ("ANA", β-D-arabinose) sequences were also examined. The ability of these oligomers to form triple helices was determined from ultraviolet spectroscopic melting experiments (as described in Example 3) and native gel electrophoresis, in a solution containing 100 mM sodium acetate and 1 mM ethylenediamine tetraacetate (EDTA), pH 5.5. Molar extinction coefficients for oligonucleotides were calculated from those of the mononucleotides and dinucleotides according to nearest-neighbouring approximations (Puglisi, J.D.; Tinoco, I. Jr. Methods in Enzymology, Dahlberg, J.E.; Abelson, J.N. (eds.), *180*, pp. 304, Academic Press, S.D., 1989). The values for the hybrid hairpin was assumed to be the sum of their DNA plus RNA components: DNA/DNA, 26.5; DNA/RNA, 27.1; (units= 10^4 M^{-1} cm^{-1}). The molar extinction coefficient for the oligoarabinonucleotide strands was assumed to be the same as a normal DNA strand (9.6×10^4 M^{-1} cm^{-1} units). Complexes were prepared by mixing equimolar amounts of interacting strands, e.g., **VII** + hairpin DNA/DNA or DNA/RNA, and lyophilizing the resulting mixture to dryness. The resulting pellet was then re-dissolved in 100 mM NaOAc/ 1 mM buffer (pH 5.5). The final concentration was 2 μM in each strand. The solutions were then heated to 80 °C for 15 min, cooled slowly to r.t., and stored at 4 °C overnight before measurements. Prior to the thermal run, samples were degassed by placing them in a speed-vac concentrator (2 min). Denaturation curves were acquired at 260 nm at a rate of heating of 0.5 °C/min. Melting temperatures (T_m) were calculated from the first derivative of the melting curves. The results of the melting experiments is shown in FIGURE EX4-1.

The results show that when **VII** was mixed with an equimolar concentration of hairpin DNA/DNA (**VIII**) or DNA/RNA (**IX**), a biphasic transition was observed upon heating the solution from 10 °C to 90 °C. The low temperature transition is assigned to the dissociation of **VII** from the

target hairpins (Roberts, R.W.; Crothers, D.M. *Science* 1992, *258*, 1463). The high temperature transition corresponds to the melting of the hairpin duplexes since it was also observed when a solution of hairpin duplex alone was heated under identical conditions (see FIGURE EX4-1). The data show that T_m values for low temperature transitions resulting from mixtures of **VII** (2'-deoxy-2'-fluoro-β-D-arabinose) + hairpins are considerable higher than T_m values for transitions from the corresponding ANA (β-D-arabinose) + hairpin, or DNA (2'-deoxy-β-D-ribose) + hairpin mixtures. For example, as can be seen from the melting curves shown in FIGURE EX4-1, the T_m for the dissociation of strand **VII** from the DNA/DNA hairpin (**VIII**) is 49 °C, compared to 34 °C and 40 °C, for the dissociation of the ANA (β-D-arabinose; not shown) and DNA (2'-deoxy-β-D-ribose) oligonucleotides, respectively. Similarly, the first transition for the triplex formed by **VII** and DNA/RNA hairpin (**IX**) was 53 °C, compared to 43 °C and 45 °C, for the corresponding triplexes formed by the ANA (β-D-arabinose; not shown) and DNA (2'-deoxy-β-D-ribose) strands, respectively.

The equilibrium between single-, double-, and triple-stranded species of **VII** + hairpin mixtures was also directly monitored by polyacrylamide gel electrophoresis (FIGURE EX4-2). This method provides a convenient way to monitor triplex formation and to qualitative check on the stoichiometry of interaction the strands (Kibler-Herzog, L. *et al. Nucleic Acids Res.* 1990, *18*, 3545). The results in FIGURE EX4-2 show that the **VII** strand, hairpins, and triple-helical complexes can be separated with excellent resolution on a polyacrylamide gel at low temperature. As can be seen from the gel results, the triple-helical complex is nearly quantitatively formed at a 1:1 molar ratio of **VII**:hairpin. This is in contrast to the incubation of ANA and hairpin (1:1 molar ratio) which, under the same conditions, gave a mixture of ANA + hairpin + triplex (see Noronha, A.; Damha, M.J. *Nucleic Acids Res.* 1998, *26*, 2665). This is in complete agreement with the T_m results which indicates that the **VII** (2'-deoxy-2'-fluoro-β-D-arabinose) strand has a significantly higher affinity for the DNA/DNA and DNA/RNA hairpin duplexes relative to the ANA (β-D-arabinose) strand.

EXAMPLE 5

Induction of Ribonuclease H (RNAseH) Activity by Oligonucleotides possessing β-D-Arabinose as Sugar Component

Defined-sequence oligonucleotides, 18-units in length, were used in these experiments, i.e.,

5'-d(AGCTCCCAGGCTCAGATC) –3'	"DNA"
5'-ara(AGCUCCCAGGCUCAGAUC)-3'	"ARA" (i.e., II)
5'-ribo(AGCUCCCAGGCUCAGAUC)-3'	"3',5'-RNA"
5'-ribo(AGCUCCCAGGCUCAGAUC)-3'	"2',5'-RNA"

These oligomers are complementary to a sequence within the R region of HIV-1 genomic RNA. The target RNA used was a synthetic 18 nt 3',5'-RNA oligonucleotide, identical to the sequence within the HIV R region, and exactly complementary to the sequence of the above oligonucleotides.

The ability of the above oligonuclcotides to elicit RNaseH degradation of target RNA was determined in assays (10 μL final volume) that comprised 5 pmol of 5'-[^{32}P]- target RNA and 15 pmol of test oligonucleotide in 60 mM Tris-HCl (pH 7.8, 25°C) containing 2mM dithiothreitol, 60 mM KCl, and either 10 mM $MgCl_2$ or 0.1 mM $MnCl_2$. Reactions were started by the addition of HIV-RT or *E. Coli* RNaseH. Incubations were carried out at 25°C for varying times (generally 20 to 30 minutes). Reactions were quenched by the addition of loading buffer (98% deionized formamide containing 10 mM EDTA and 1mg/mL each of bromophenol blue and xylene cyanol), and heating at 100°C for 5 minutes. The reaction products were resolved by electrophoresis using a 16% polyacrylamide sequencing gel containing 7 M urea, and visualized by autoradiography. The result of such experiments is shown in FIGURE EX5.

The results show that the oligonucleotide based on 2'-deoxyribose ("DNA") or β-D-arabinose ("ARA") are able to form duplexes with target RNA that serve as substrates for the RNase H activity of

either HIV-1 RT or *E. coli* RNase H, as indicated by the numerous smaller sized degradation products of the target RNA in FIGURE EX5. This RNase H degradation was noted with either Mn^{2+} (illustrated) or Mg^{2+} (not shown) as metal. In contrast, oligonucleotides based on D-ribose, either in 3',5'-linkages (3',5'-RNA), or in 2',5'-linkages (2',5'-RNA) were unable to elicit this RNase H degradation of target RNA, even though these test oligonucleotides were competent to form duplexes with the target RNA. Similarly, an oligonucleotide based on β-D-2'-deoxyribose, but of a random base sequence (DNA random) not complementary to the target RNA (and therefore unable to form duplexes), was also unable to elicit RNase H activity.

EXAMPLE 6

Induction of Ribonuclease H (RNAseH) Activity by Oligonucleotides possessing 2'-Deoxy-2'-Fluoro-β-D-Arabinose as Sugar Component

Homopolymeric octadecanucleotides with either thymine (T) or uracil (U) as base component were used in these experiments. The target RNA used was a synthetic 3',5'-linked rA_{18} oligonucleotide, exactly complementary to the sequence of the test oligonucleotides.

The ability of oligonucleotides with 2'-deoxy-2'-fluoro-β-D-arabinose as sugar component, and other oligonucleotides, to elicit RNaseH degradation of target RNA was determined in assays (10 μL final volume) that comprised 1 pmol of 5'-[^{32}P]- target RNA and 8 pmol of test oligonucleotide in 60 mM Tris-HCl (pH 7.8, 25°C) containing 2mM dithiothreitol, 60 mM KCl, and 2.5 mM $MgCl_2$. Reactions were started by the addition of HIV-RT or *E. Coli* RNaseH. Incubations were at 22°C for 20 minutes. Reactions were quenched by the addition of loading buffer (98% deionized formamide containing 10 mM EDTA and 1mg/mL each of bromophenol blue and xylene cyanol, and heating at 100°C for 5 minutes. The reaction products were resolved by electrophoresis using a 16%

polyacrylamide sequencing gel containing 7 M urea, and visualized by autoradiography. The result of such an experiment is shown in FIGURE EX6.

Each of the oligonucleotides based on β-D-2'-deoxyribose with phosphodiester bonds (*i.e.*, PO-dT_{18}, abbreviated as "dT"), β-D-2'-deoxyribose with phosphorothioate bonds (PS-dT_{18}, or "SdT"), 2'-deoxy-2'-fluoro-β-D-arabinose (PO-2'-F-araT_{18}, or "aFT"), β-D-ribose (PO-rU_{18}, or "rU") and 2'-deoxy-2'-fluoro-β-D-ribose (PO-2'-F-rT_{18}, or "rFT") were able to form duplexes with target RNA (rA_{18}). Only duplexes formed with oligonucleotides "dT", "SdT" or "aFT" served as substrates for the RNase H activity of either HIV-1 RT or *E. coli* RNase H, as indicated by the numerous smaller sized degradation products of the target RNA in FIGURE EX6. Duplexes formed with rFT or rU could not serve as substrates for the RNase H activity of either HIV-1 RT or *E. coli* RNase H. Under these conditions an octadecanucleotide based on β-D-arabinosyluracil (PO-araU_{18}, or "aU") was unable to form a duplex with target rA, and accordingly was unable to elicit RNase H activity (these findings are consistent with those reported by Giannaris and Damha, who found that araU_8 was unable to form a duplex with poly rA; Giannaris, P.A.; Damha, M.J., *Can.J.Chem.* 1994, *74*, 909).

EXAMPLE 7

Nuclease Stability of Oligoarabinonucleotides

Thymine octadecanucleotides based on 2'-deoxyribose with phosphodiester bonds (i.e., PO-dT_{18}, abbreviated as "dT") and 2'-deoxy-2'-F-β-D-arabinose (PO-2'-F-araT_{18}, or "aFT") were compared for stability against degradation by serum nucleases and cellular nucleases.

Stability against serum nucleases was assessed by adding 1 pmol of 5'-[^{32}P]-ODN to a reaction assay (10 μL final volume) containing 90% horse serum. Reactions were incubated at 20°C for varying times, then aliquots were removed and diluted with gel loading buffer (98% deionized formamide

containing 10 mM EDTA and 1 mg/mL each of bromophenol blue and xylene cyanol), boiled for 5 minutes then resolved by electrophoresis on a 16% polyacrylamide sequencing gel containing 7 M urea. Separated products were visualized by autoradiography.

The results of such an experiment is shown in FIGURE EX7. The "aFT" oligonucleotide was substantially more resistant to degradation by serum nucleases, as indicated by the decreased number of smaller molecular size degradation products compared to that noted with normal "dT" oligonucleotide. Under the same conditions, oligonucleotides based on β-D-2'-deoxyribose with phosphorothioate bonds (PS-dT_{18}, or "SdT") were virtually unaffected by serum nucleases (data not shown), as previously established by many investigators.

Unfractionated mouse liver crude homogenates (prepared by homogenizing mouse livers in an equal volume of 20 mM Tris-HCl (pH 7.9, 20°C) containing 60 mM KCl, 1mM dithiothreitol and 12% (v/v) glycerol) were used as a source of cellular nucleases. Stability against cellular nucleases was assessed by adding 1 pmol of 5'-[^{32}P]-ODN to a reaction assay (10 μL final volume) containing 90% unfractionated mouse liver crude homogenate. After varying times of incubation at 20°C, aliquots were removed, diluted with gel loading buffer (98% deionized formamide containing 10 mM EDTA, 1mg/mL each of bromophenol blue and xylene cyanol), boiled for 5 minutes then resolved by electrophoresis on a 16% polyacrylamide sequencing gel containing 7 M urea. Separated products were visualized by autoradiography. The results established that the "aFT" oligonucleotide was significantly more resistant than the corresponding "dT" oligonucleotide to degradation by cellular nucleases.

EXAMPLE 8

Nonspecific Interaction of Oligoarabinonucleotides with Cellular Proteins

The ability of thymine octadecanucleotides based on β-D-2'-deoxyribose with phosphodiester bonds (*i.e.*, PO-dT_{18}, abbreviated as "dT"), β-D-2'-deoxyribose with phosphorothioate bonds (PS-dT_{18}, or ""SdT"), or 2'-deoxy-2'-fluoro-β-D-arabinose (PO-2'-F-$araT_{18}$, or "aFT") to bind nonspecifically to proteins in a mouse liver crude extract was analyzed by a gel shift assay procedure. The antisense oligonucleotides were radioactively labeled at the 5'-terminus using [γ-^{32}P]-ATP and and T4 polynucleotide kinase according to standard protocols (Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., 1994). Mouse liver crude extracts were prepared by homogenizing mouse livers in an equal volume of buffer (20 mM Tris-HCl (pH 7.9, 20°C) containing 60 mM KCl, 1mM dithiothreitol and 12% (v/v) glycerol), followed by centrifugation to remove membrane particles and cell debris.

One μL of homogenate (undiluted, or 1:10 and 1:100 dilutions) was mixed with an equal volume of buffer containing 1 pmol of ^{32}P-labeled oligonucleotide was incubated at 4°C for 10 minutes, then subjected to resolution of free and protein-bound oligonucleotide by electrophoresis on 16% non-denaturing polyacrylamide gels. The gel running buffer contained 5 mM $MgCl_2$ and lacked EDTA. Gels were run for 24 hours at 4°C, at 8V/cm. Following completion of the electrophoresis, the gels were dried and the positions of the free and protein-bound oligonucleotides visualized by autoradiography.

The results of such an experiment are shown in FIGURE EX8. Essentially all of the phosphorothioate "SdT" was bound to the extract proteins in samples at all dilutions tested (undiluted extract, 1:10 or 1:100 diluted extracts). The amount of protein binding by the corresponding phosphodiester "dT" was significantly diminished under the same conditions; the amount of nonspecific protein binding was even less for the "aFT" oligonucleotide than for the corresponding "dT" oligonucleotide. Thus, the amount of nonspecific protein binding was SdT >> dT > aFT. Addition of a 100-fold excess of cold dT over [^{32}P]-labeled "SdT" was unable to disrupt the SdT-protein complexes.

EXAMPLE 9

Effect of Oligonucleotides with 3'-Terminal β-D-Arabin se on Chain Termination by 2',3'-Dideoxynucleotides during DNA Synthesis Catalyzed by HIV-1 Reverse Transcriptase.

Oligonucleotides in which the 3'-terminal nucleotide has β-D-arabinose as sugar component are known to act as primers for DNA synthesis carried out by HIV-1 RT. However, this DNA synthesis shows significant "pausing" after incorporation of four additional deoxynucleotides (n+4) (Borkow, G. *et al. International Journal of Biochemistry and Cell Biology*, 1997, *29*, 1285).

HIV-1 RT-catalyzed DNA synthesis was carried out in the absence or in the presence of 2',3'-dideoxynucleoside 5'-triphosphate chain terminators, using a synthetic 30 nt RNA template:

3'-r(UCGAGGGUCCGAGUCUAGACGGCCAGAGGG)-5'

and synthetic 18 nt DNA primers, exactly complementary to the 18 nt region near the 3'-end of the template RNA. Two different primers were used, differing only in the sugar component of the 3'-terminal nucleotide:

5'-d(AGCTCCCAGGCTCAGATC)-3', and

5'-d(AGCTCCCAGGCTCAGAT)araC-3'

where "araC" terminus is 1-(β-D-arabinofuranosyl)cytosine.

The DNA primers were radioactively labeled at the 5'-terminus using [γ-^{32}P]-ATP and and T4 polynucleotide kinase according to standard protocols (Ausubel, F.M. *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. 1994), and mixed with an equivalent concentration of RNA transcript to allow formation of duplex template/primers (T/P).

The preformed T/P were incubated at 37°C with HIV-1 RT in 50 mM Tris-HCl (pH 7.8, 37°C) containing 60 mM KCl, 10 mM dithiothreitol and 10 mM $MgCl_2$, and DNA synthesis was initiated by the addition of dATP+dCTP+dGTP+dTTP to a final concentration of 25 μM each. In other reactions, DNA synthesis was initiated by the addition of dATP+dCTP+dGTP+dTTP to a final concentration of 25 μM each, plus the addition of ddCTP to a final concentration of 10 μM. After varying times of incubation, samples were quenched by the addition of gel loading buffer (98% deionized formamide containing 10 mM EDTA, 1mg/mL each of bromophenol blue and xylene cyanol), boiled for 5 minutes then resolved by electrophoresis on a 16% polyacrylamide sequencing gel containing 7 M urea. Separated products were visualized by autoradiography.

The results, illustrated in FIGURE EX9-1, imply that the amount of chain termination by incorporation of ddCMP at position n+4 is increased in reactions primed by the oligonucleotide possessing a β-D-arabinose sugar at the 3'-end compared to those in reactions primed by the oligonucleotide with a normal β-D-2'-deoxyribose sugar at the 3'-end. These differences in ddCMP incorporation are perhaps better illustrated in the graph presented as FIGURE EX9-2.

TABLE 1: Base composition, yield and molecular masses of oligoarabinonucleotides

Base sequence of Oligonucleotide (5'-to-3')		Yield (A-260 units)	MW: MALDI [M:Na+] found (± 0.1%) / expected
a(AGC UCC CAG GCU CAG AUC)	(II)	5	5648 / 5653
a(AAA AAA AAA AAA AAA AAA)	(III)	9.5	5880 / 5860
a(UUU UUU UUU UUU UUU UUU)	(IV)	8.5	5449 / 5446

TABLE 2: Base composition, yield and molecular masses of 2'-F-oligoarabinonucleotides

Base sequence of 2'-F-Oligoarabinonucleotide (5'-to-3')		Yield (A-260 units)	MW: MALDI [M:Na+] found (± 0.1%) / expected
TTT TTT TTT TTT TTT TTT	(V)	14.4	5713 / 5717
AAA AAA AAA AAA AAA AAA	(VI)	27	5898 / 5897
CCT CTC CTC CCT	(VII)	14	3682 / 3683

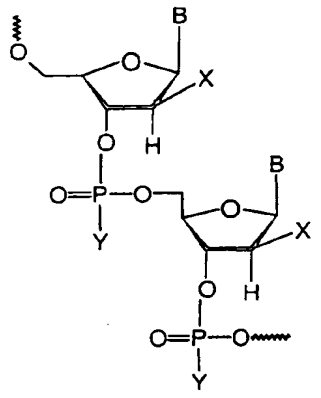
CLAIMS

We claim,

1. A method for hybridizing an oligonucleotide comprising arabinose sugars to single stranded RNA, in a sequence specific manner, in order to induce RNase H activity and cleave said RNA.

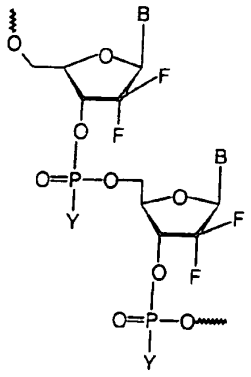
2. A method for hybridizing an oligonucleotide comprising arabinose sugars to duplex DNA, or hybrid DNA (purine)/ RNA (pyrimidine), in a sequence specific manner, in order to inhibit DNA replication and/or DNA transcription.

3. The method of claims 1 and 2 wherein said oligonucleotide has the formula:



wherein B is selected from the group consisting of adenine, guanine, uracil, thymine, cytosine, inosine, and 5-methylcytosine. X at the 2' position of the sugar ring is selected from the group consisting of a halogen (fluorine, chlorine, bromine, iodine), hydroxyl, alkyl, allyl, amino, aryl, alkoxy, and azido. Y at the internucleotide phosphate linkage is selected from the group consisting of oxygen, sulfur, methyl, amino, alkylamino, dialkylamino (the alkyl group having one to about 20 carbon atoms), methoxy, and ethoxy.

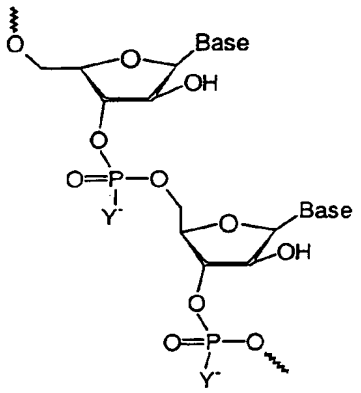
4. The methods of claim 1 and 2 wherein said oligonucleotide is



wherein B is selected from the group consisting of adenine, guanine, uracil, thymine, cytosine, inosine, 5-methylcytosine. Y at the internucleotide phosphate linkage is selected from the group consisting of oxygen, sulfur, methyl, amino, alkylamino, dialkylamino (the alkyl group having one to about 20 carbon atoms), methoxy, and ethoxy.

5. The method of claims 1 and 2 wherein said oligonucleotide is chemically modified at least at one site with ligands such as cell surface receptors, any pharmacological agent or analog to enhance one or a combination of the following: (a) its permeability into cells, (b) nuclease stability, (c) binding strength of hybridization to complementary sequences.

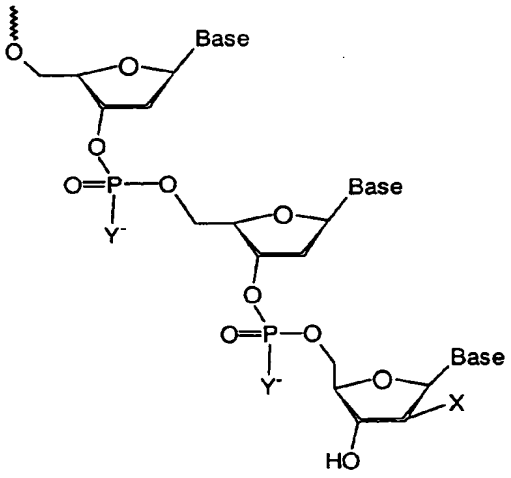
6. The method of selectively hybridizing an oligonucleotide comprising β-D-arabinofuranose nucleotide units to RNA, wherein such oligonucleotide does not hybridize to single stranded DNA in a sequence specific manner. Said oligonucleotide has the formula:



wherein the oligomer has a mixed base composition. B is selected from the group consisting of adenine, guanine, uracil, thymine, cytosine, inosine, and 5-methylcytosine. Y at the internucleotide phosphate linkage is selected from the group consisting of oxygen, sulfur, methyl, amino, alkylamino, dialkylamino (the alkyl group having one to about 20 carbon atoms), methoxy, and ethoxy.

7. A method involving hybridization of an oligonucleotide with a terminal arabinose sugar to single stranded nucleic acids (DNA or RNA), in a sequence specific manner, such that that oligonucleotide comprising arabinose sugars is recognized by and used for the initiation of DNA synthesis ("priming" activity).

8. The method of claim 7 wherein said oligonucleotide has the formula:



wherein B is selected from the group consisting of adenine, guanine, uracil, thymine, cytosine, inosine, and 5-methylcytosine. X at the 2' position of the 3'-terminal arabinose sugar ring is selected from the group consisting of a halogen (fluorine, chlorine, bromine, iodine), hydroxyl, alkyl, allyl, amino, aryl, alkoxy, and azido. Y at the internucleotide phosphate linkage is selected from the group consisting of oxygen, sulfur, methyl, amino, alkylamino, dialkylamino (the alkyl group having one to about 20 carbon atoms), methoxy, and ethoxy.

9. The method of claim 7 in combination with the administration of 2',3'-dideoxynucleosides, including but not limited to 3'-azido-3'-deoxythymidine (AZT; zidovudine) and 3'-deoxy-3'-thiacytidine (3TC) such that incorporation of said 2',3'-dideoxynucleosides into nascent DNA is enhanced, leading to improved inhibition of DNA synthesis.

10. The method of claims 7 and 9 as applied to the inhibition of reverse transcription and thereby the replication of retroviruses such as the human immunodeficiency virus.

11. The method of claims 7 and 9 as applied to the inhibition of reverse transcription and thereby the replication of hepadnaviruses such as hepatitis B virus.

12. The method of claims 7 and 9 as applied to the inhibition of prokaryotic cell DNA replication and thereby the proliferation of cells including but not limited to pathogenic bacteria causing human disease.

13. The method of claims 7 and 9 as applied to the inhibition of eukaryotic cell DNA replication and thereby the proliferation of cells including but not limited to human cancer cells.

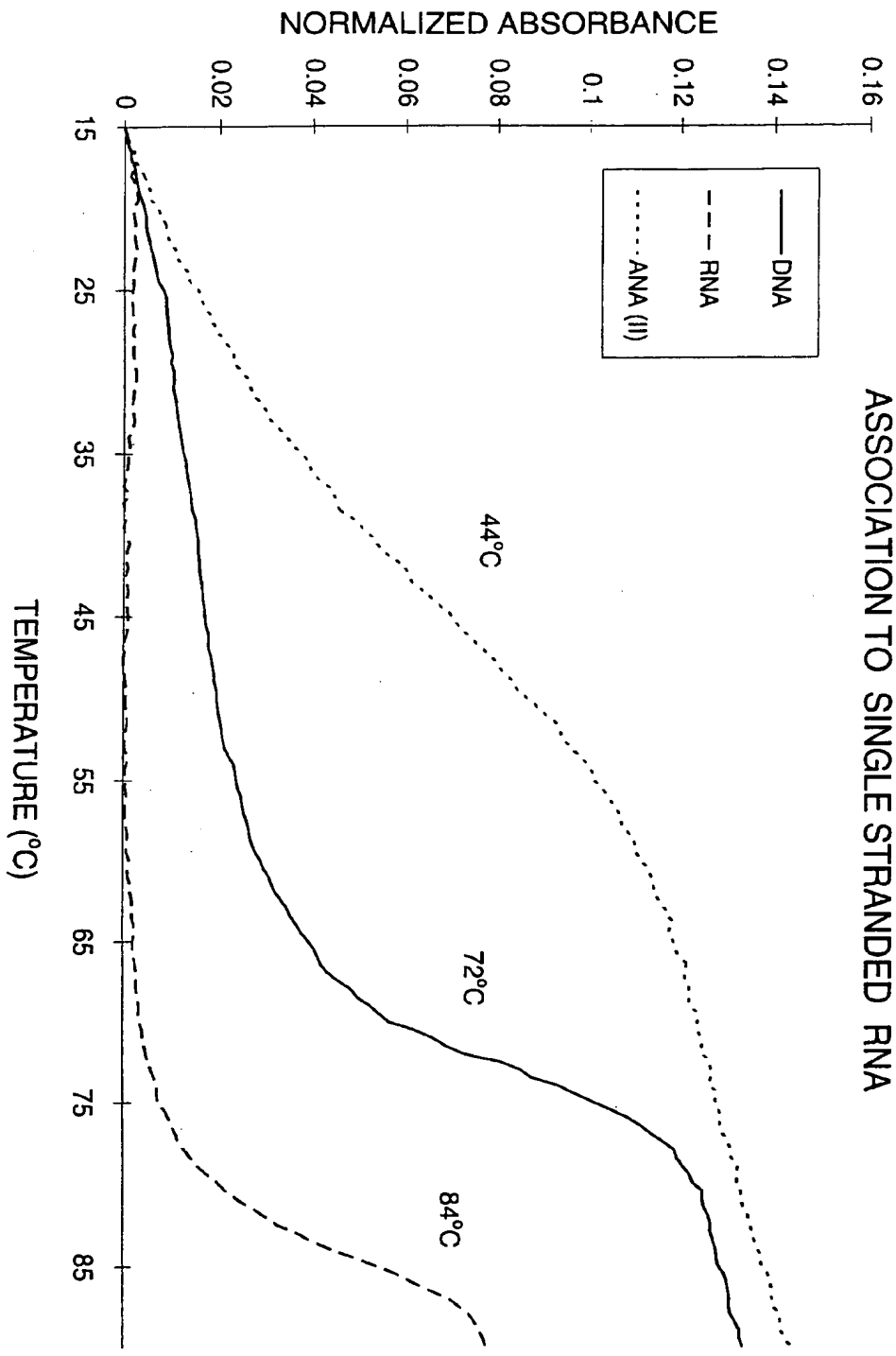


FIGURE EX3-1 (A)

ASSOCIATION TO SINGLE STRANDED RNA

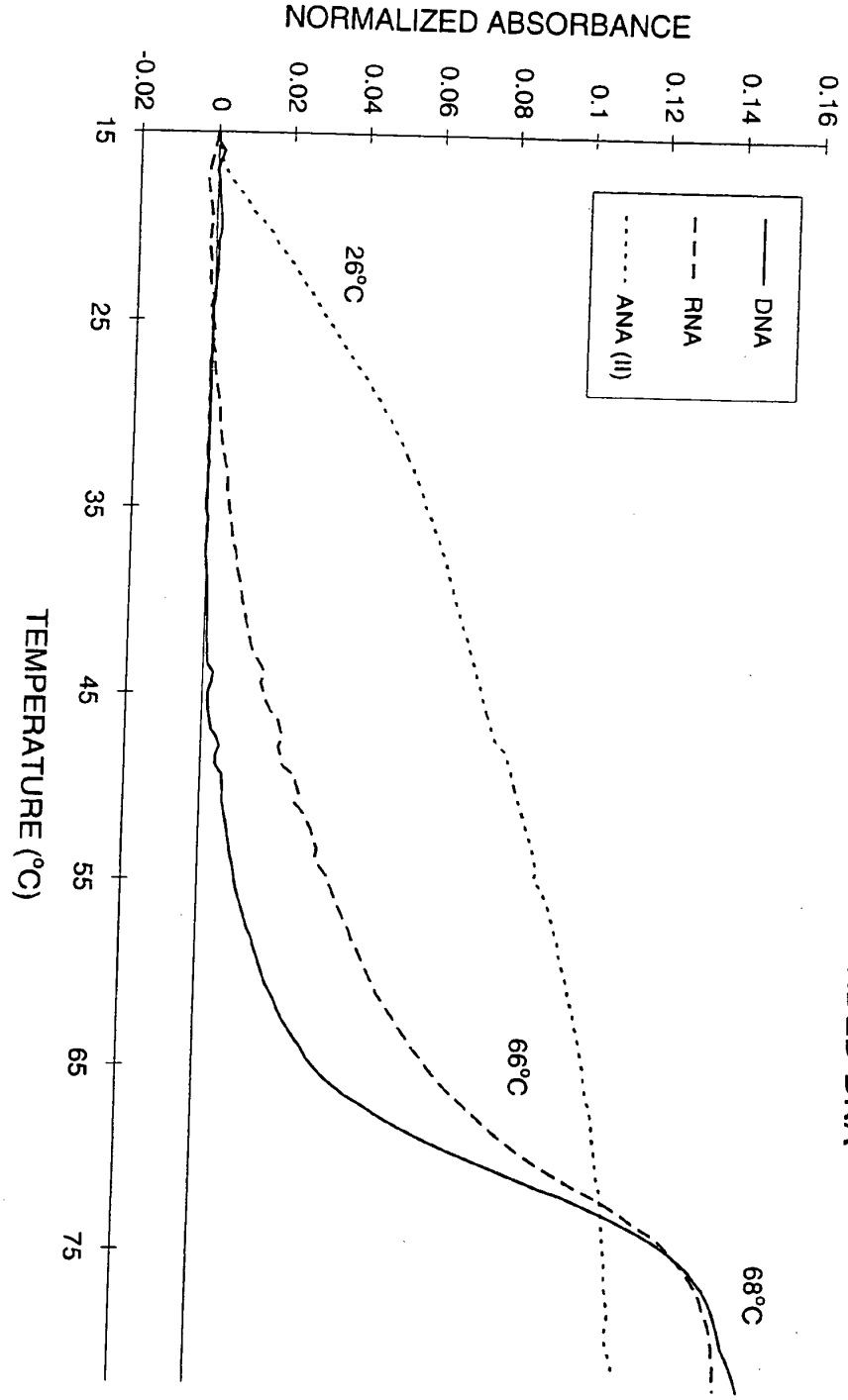
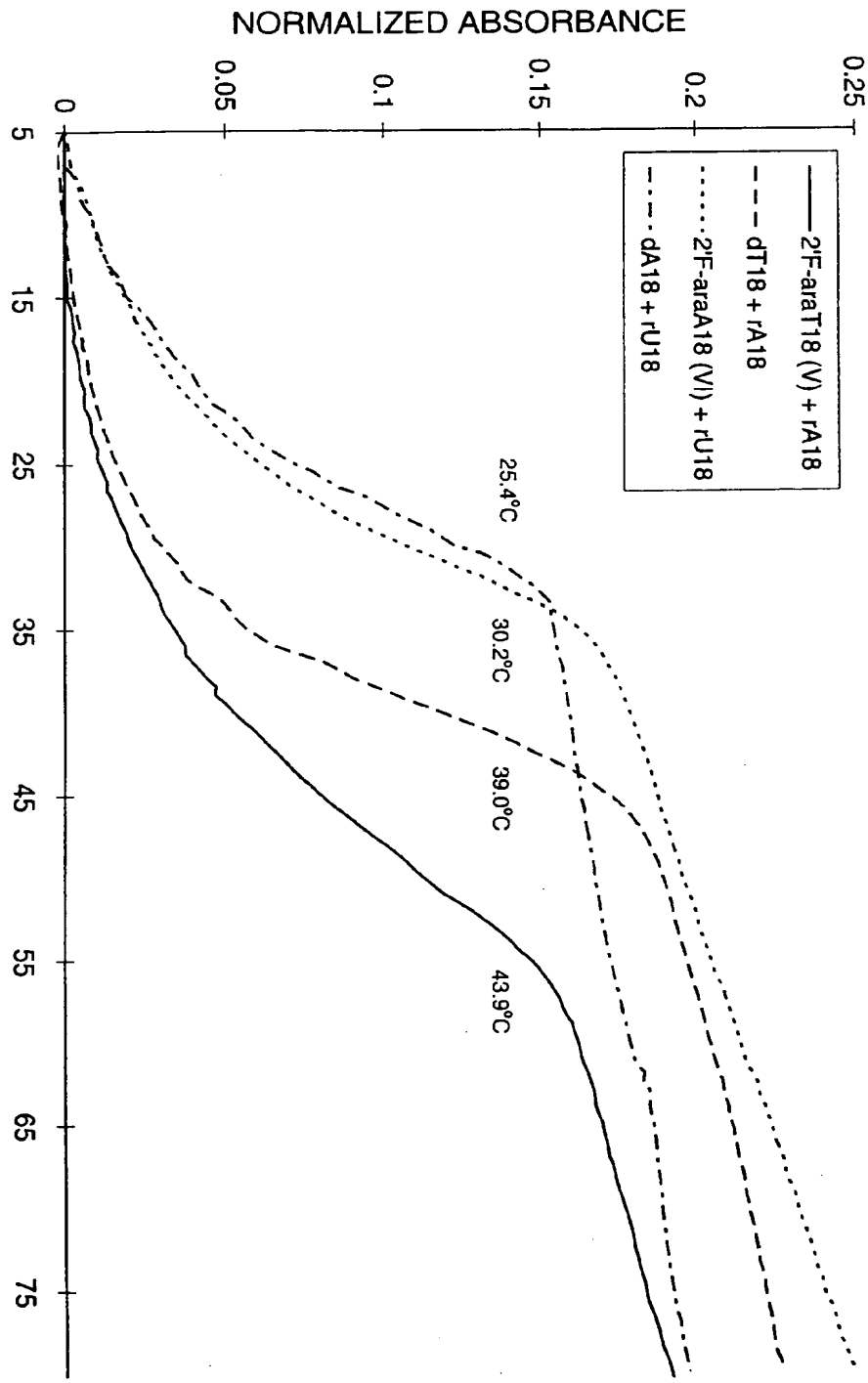


FIGURE EX3-1 (B)

ASSOCIATION TO SINGLE STRANDED DNA

FIGURE EX3-1 (C)
ASSOCIATION TO SINGLE STRANDED RNA



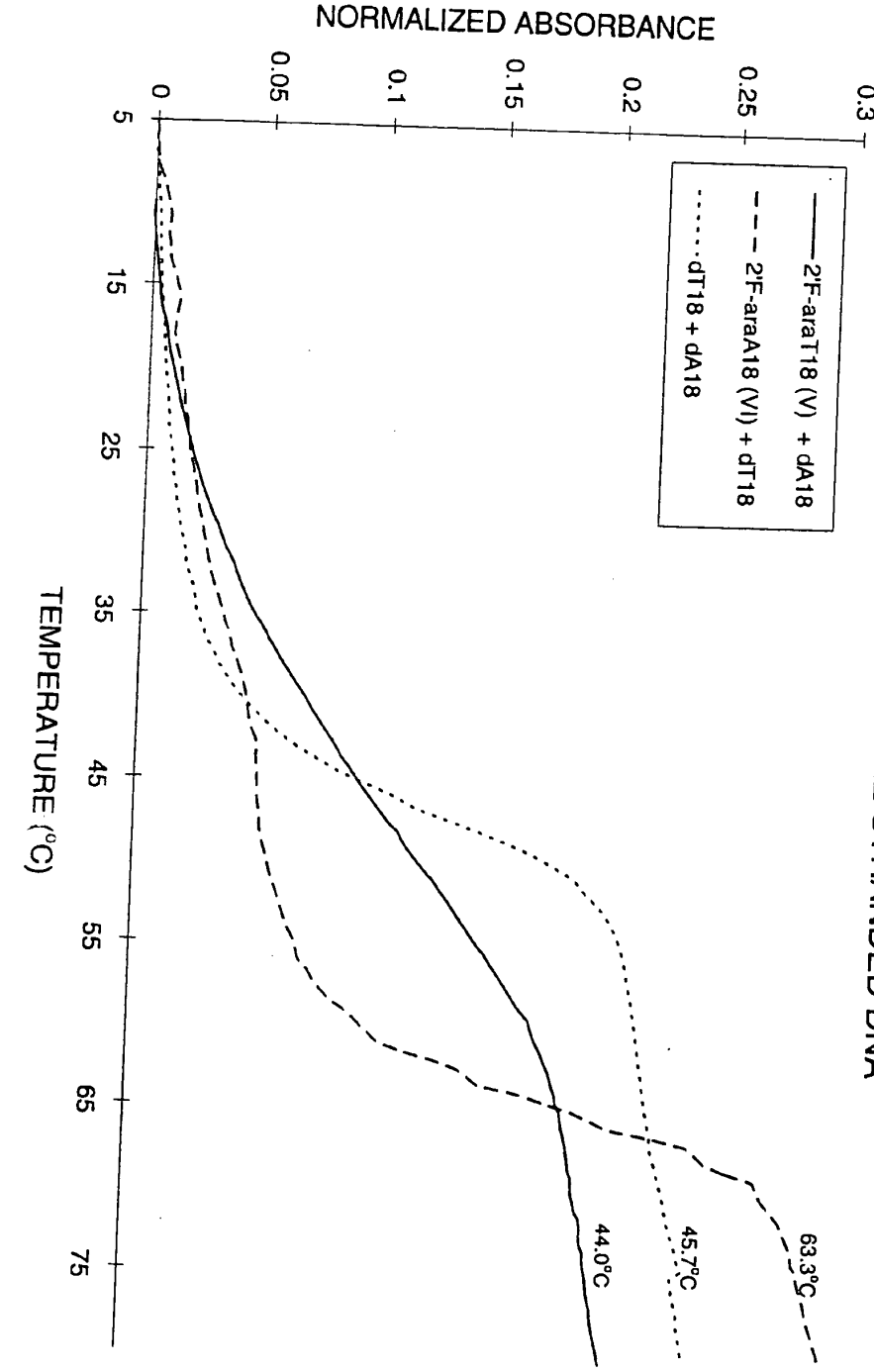


FIGURE EX3-1 (D)
ASSOCIATION TO SINGLE STRANDED DNA

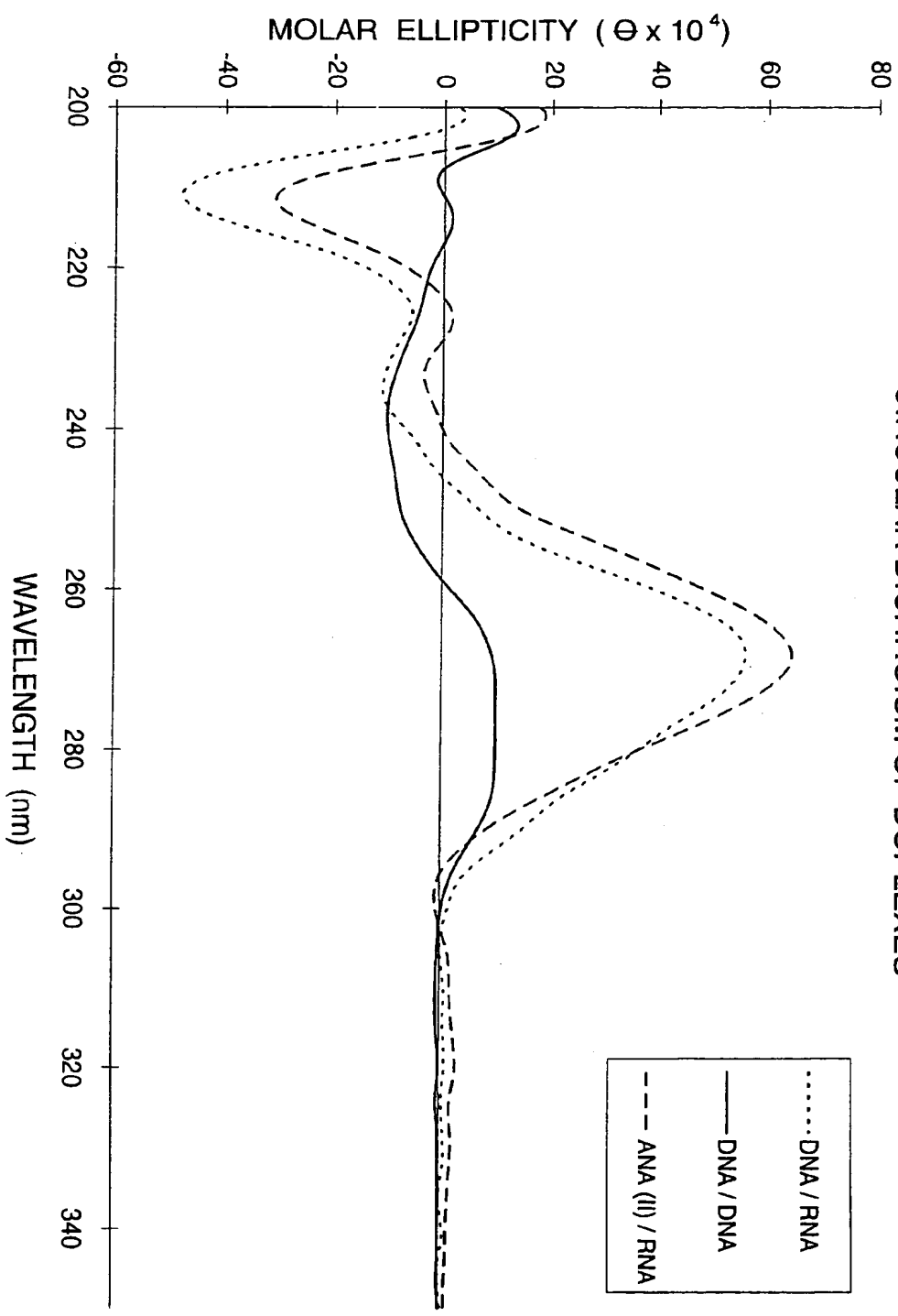


FIGURE EX3-2 (A)
CIRCULAR DICHROISM OF DUPLEXES

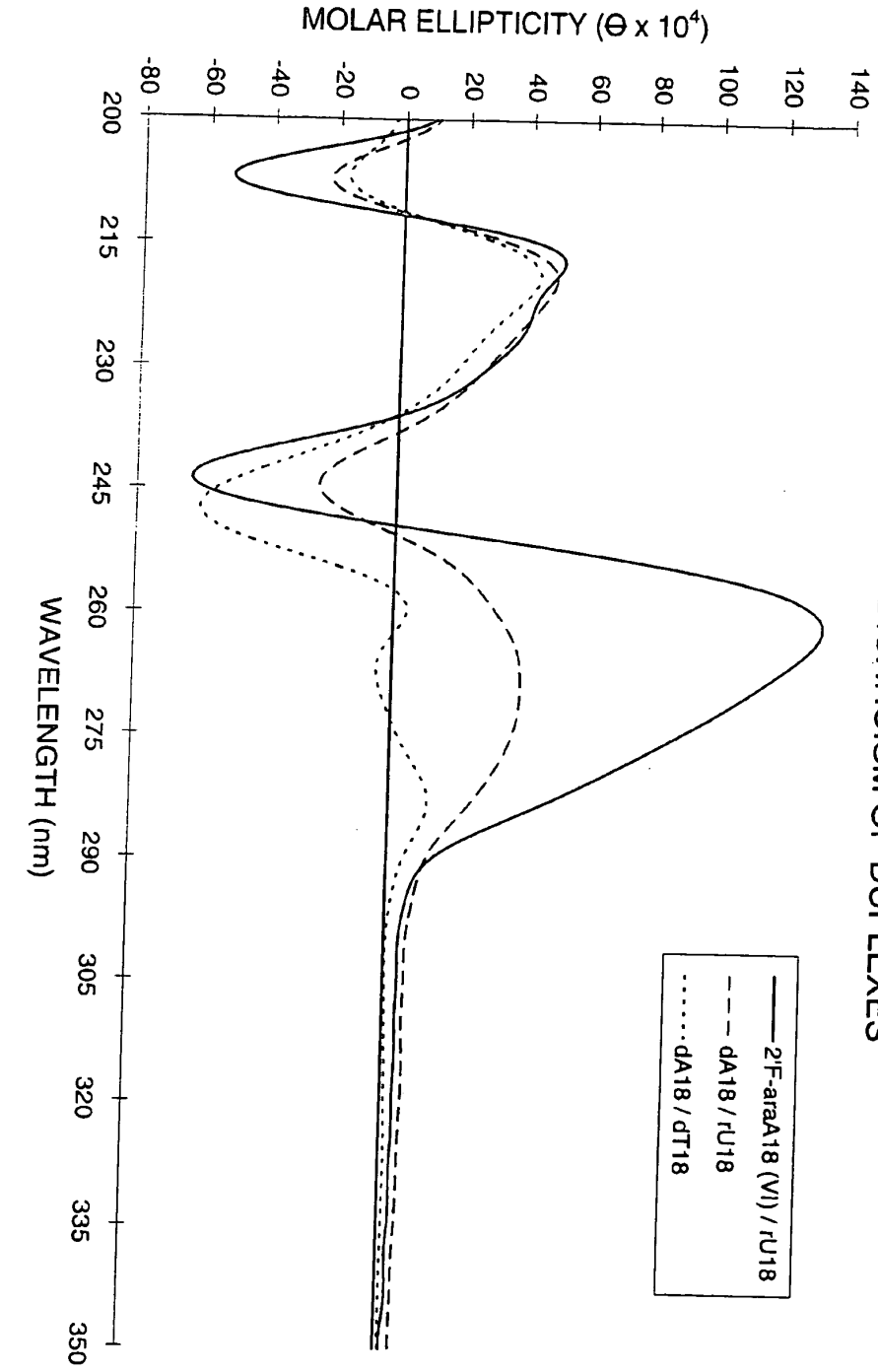
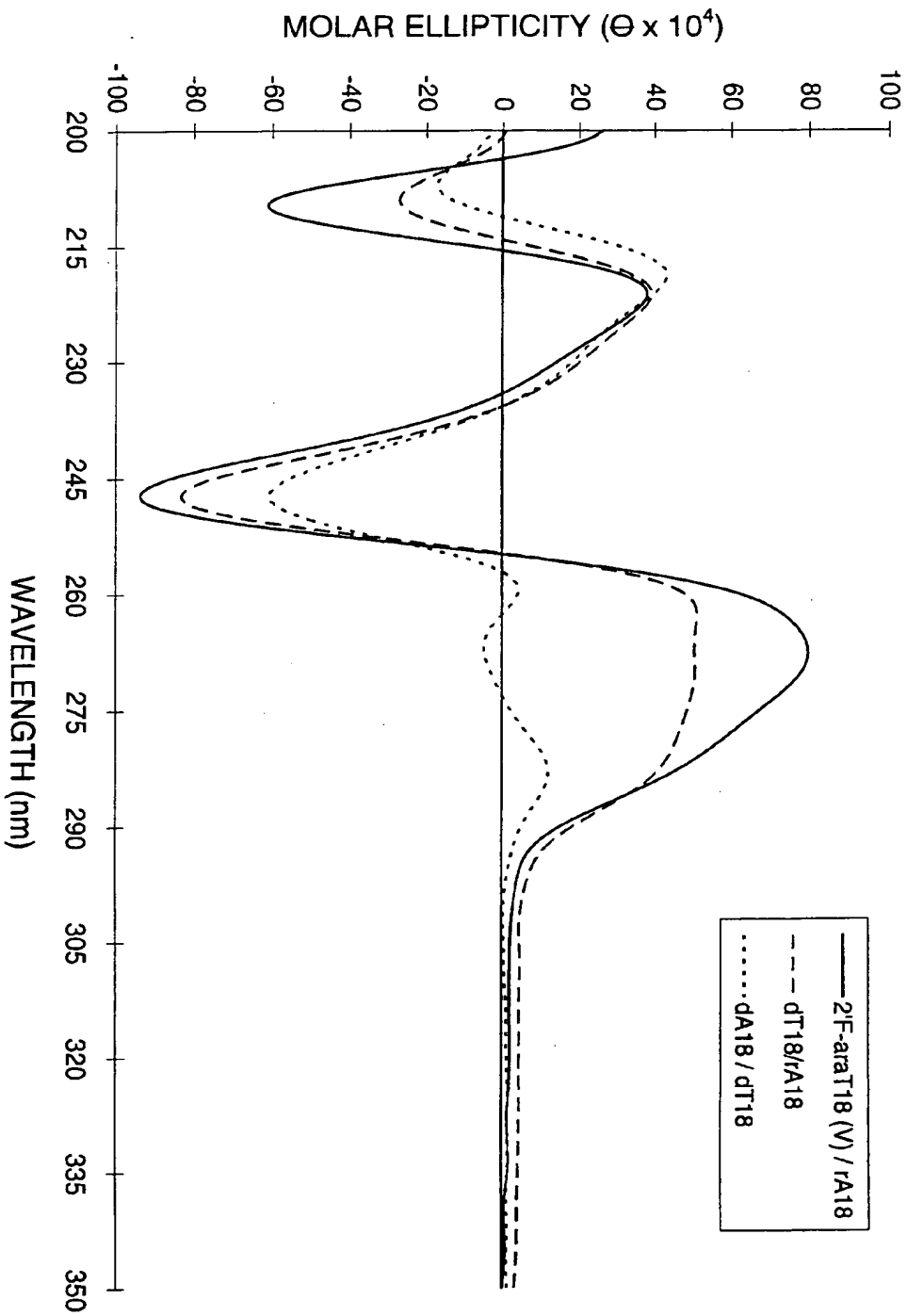


FIGURE EX3-2 (B)
CIRCULAR DICHROISM OF DUPLEXES

FIGURE EX3-2 (C)
CIRCULAR DICHROISM OF DUPLEXES



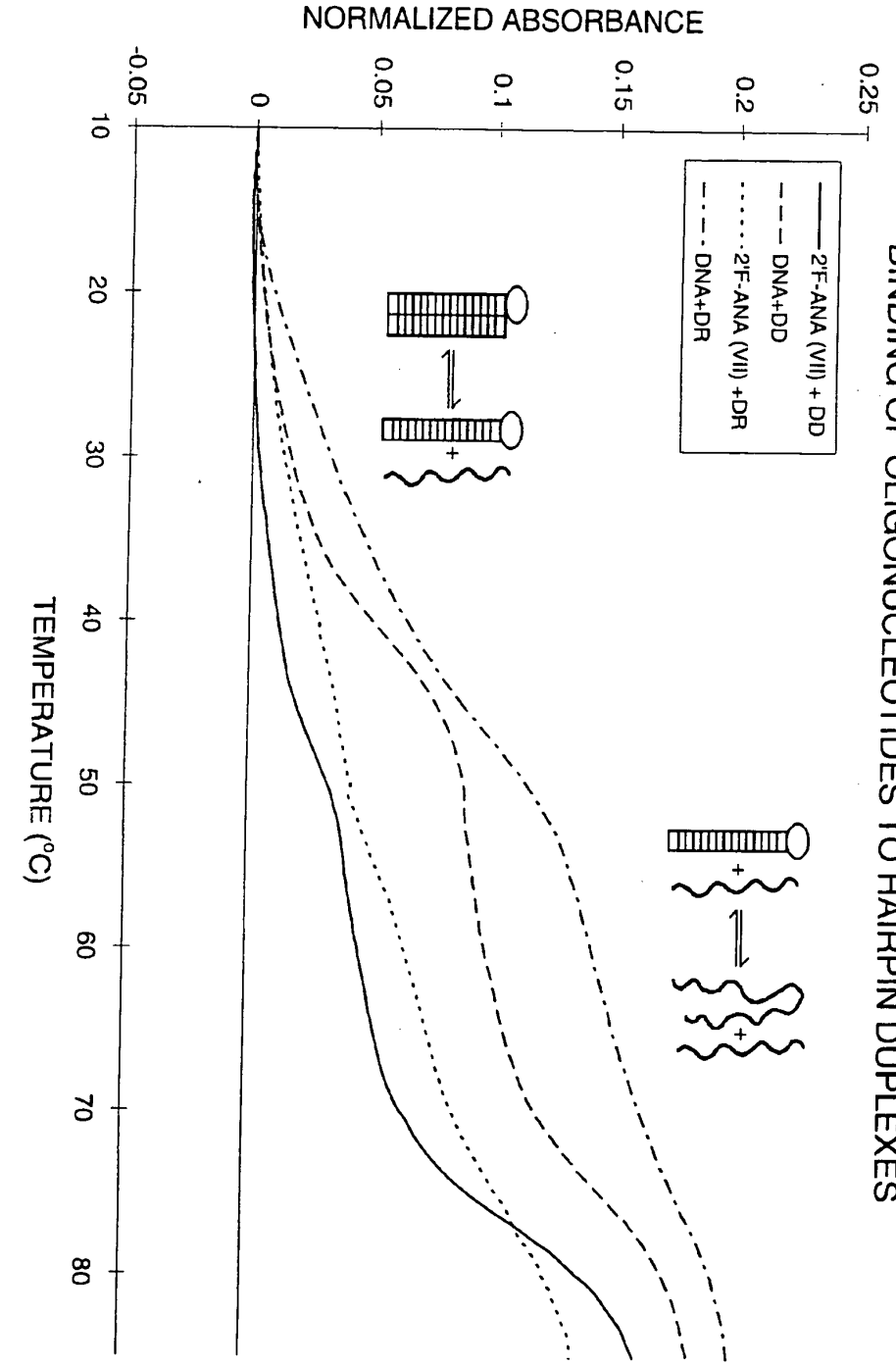
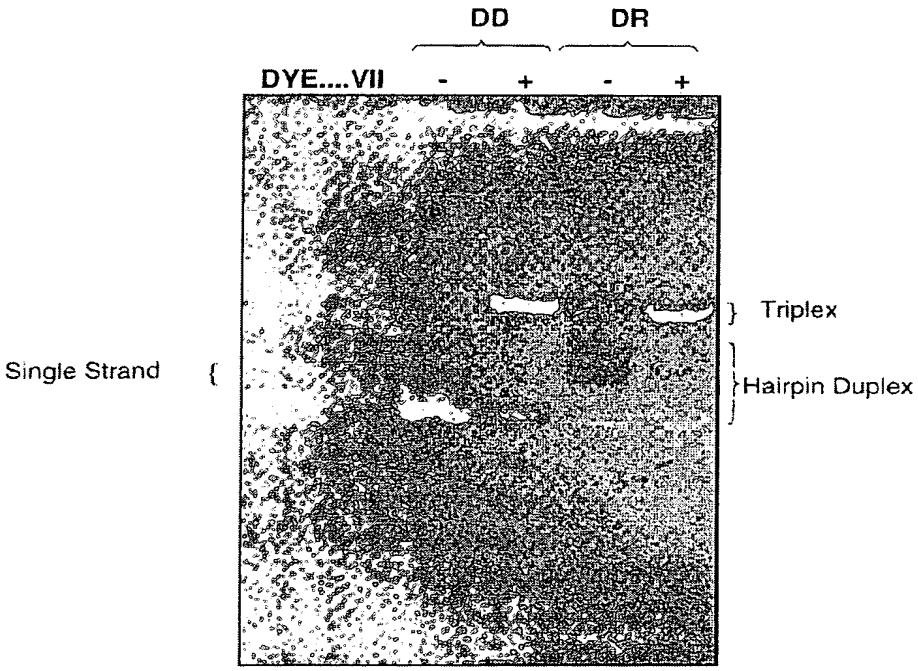


FIGURE EX4-1

BINDING OF OLIGONUCLEOTIDES TO HAIRPIN DUPLEXES

FIGURE EX4-2

GEL MOBILITY SHIFT TRIPLEX ASSAY



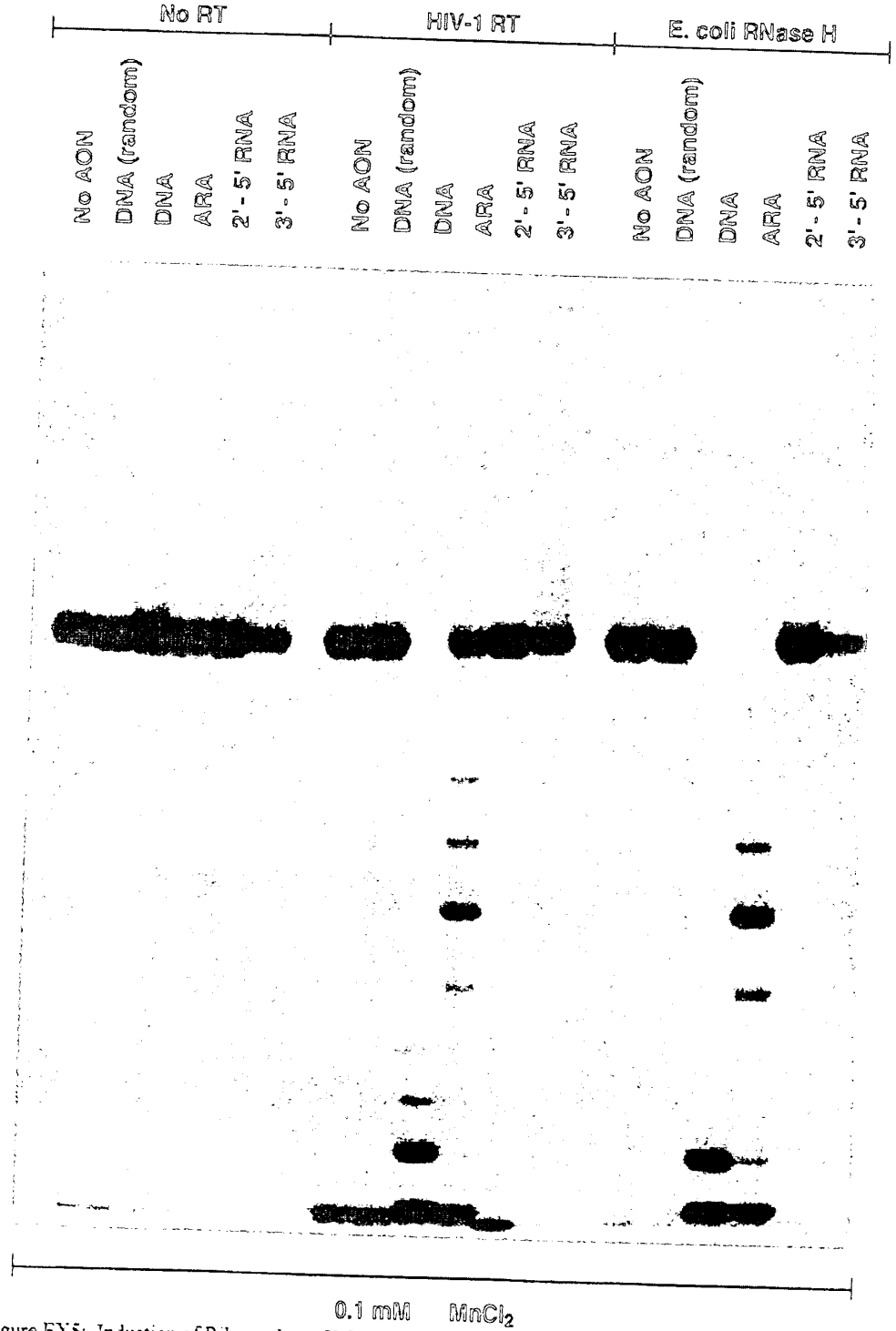


Figure EX5: Induction of Ribonuclease H (Rnase H) Activity of the enzymes HIV-1 RT and *E. coli* RNaseH by Oligonucleotides

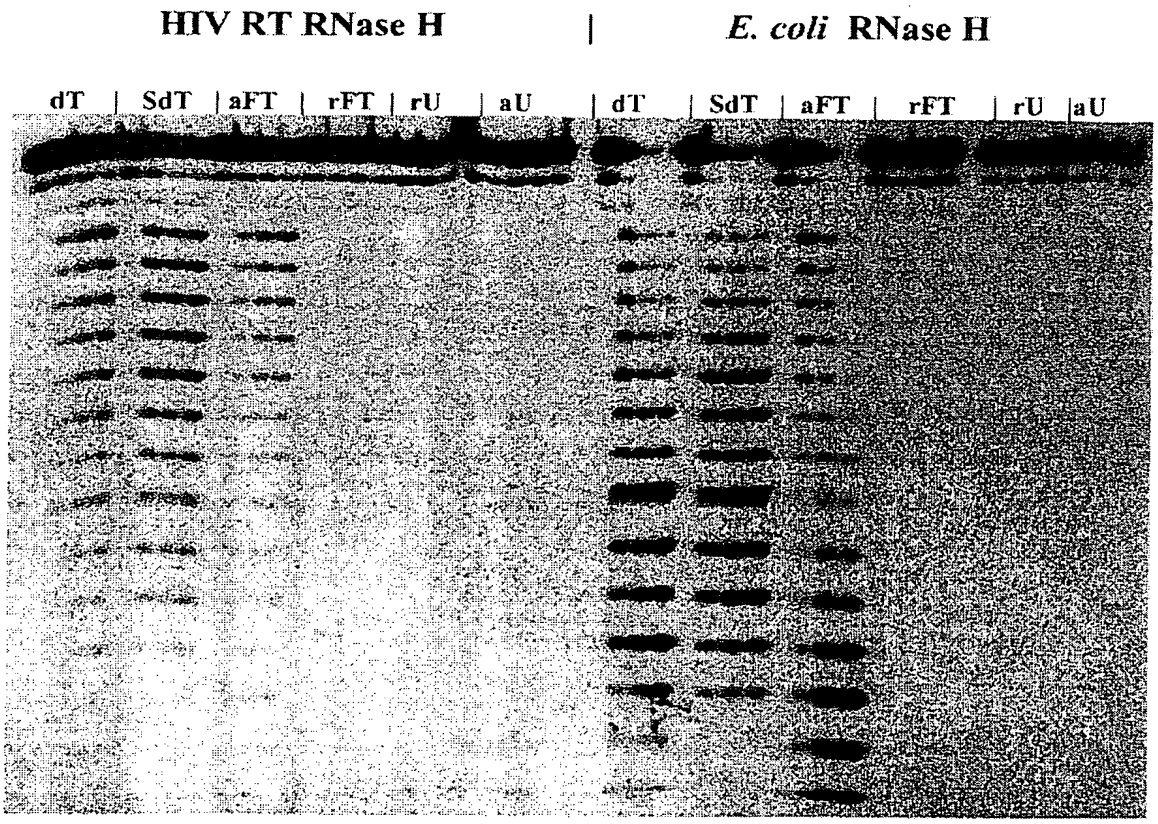


Figure EX6: Induction of Ribonuclease H (RNaseH) Activity by Oligonucleotides Possessing 2'-deoxy-2'-fluoro-β-D-Arabinose as Sugar Component.

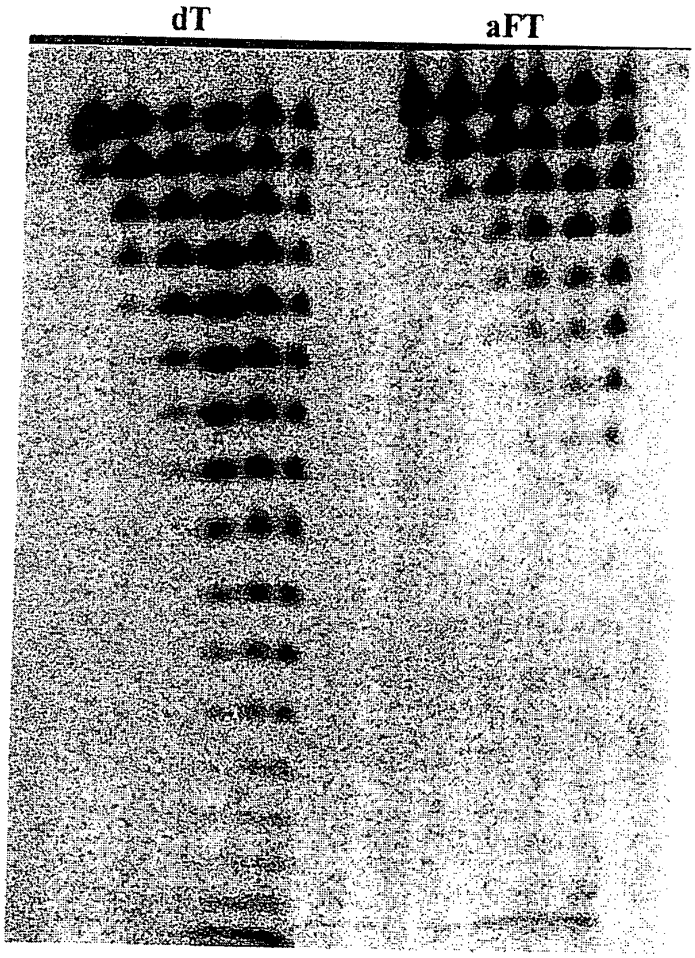


Figure EX7: Serum Nuclease Stability of Oligonucleotides with 2'-deoxy-2'-F-β-arabinose as sugar component.

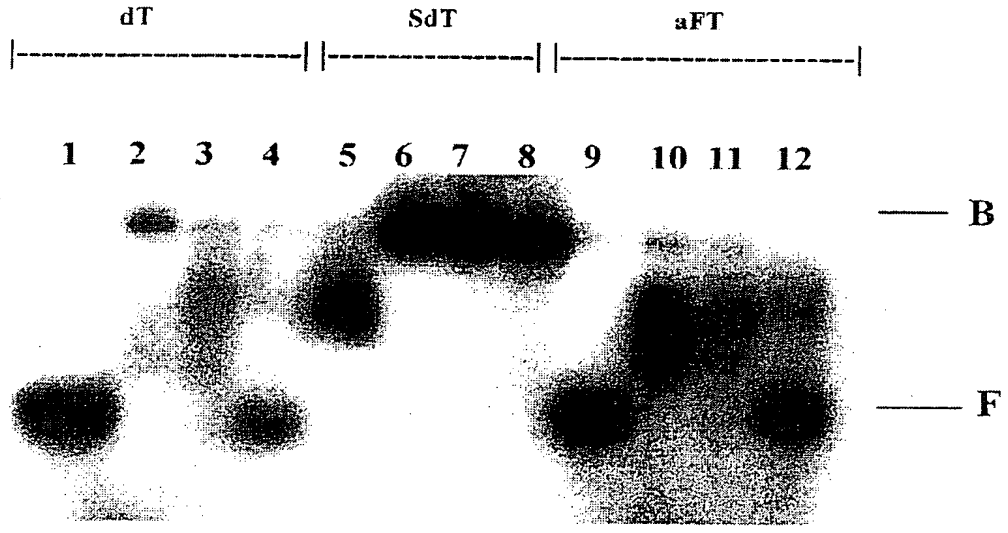


Figure EX8: Nonspecific Interaction of Oligoarabinonucleotides with Cellular Proteins.

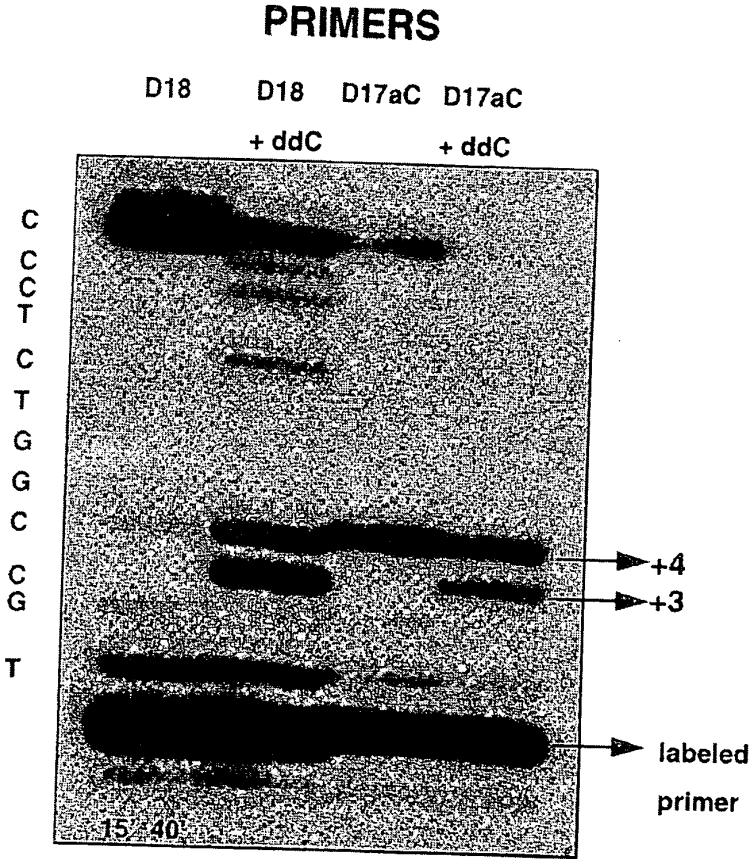


Figure EX9-1: Effect of oligonucleotides with 3'-terminal β-D-arabinose on chain termination by 2',3'-dideoxycytidine during DNA synthesis catalyzed by HIV-1 reverse transcriptase.

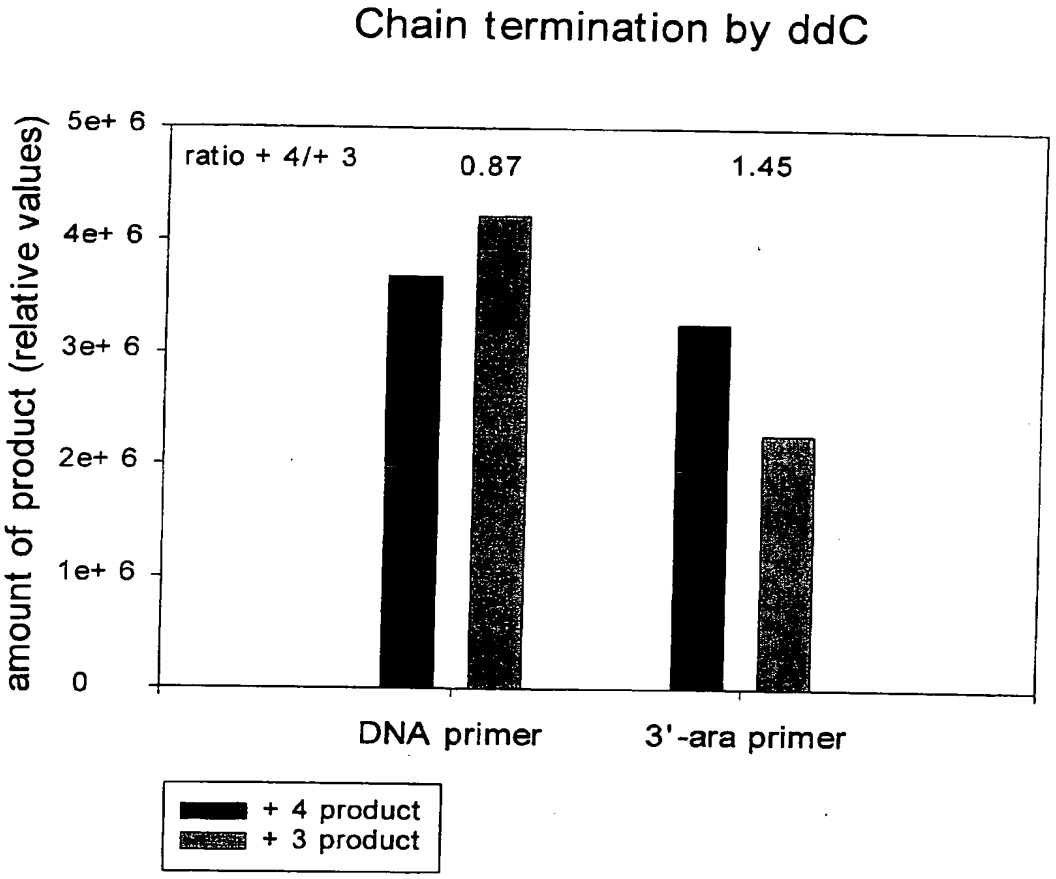


Figure EX9-2: Ratio of chain-termination products at n+4 compared to n+3.